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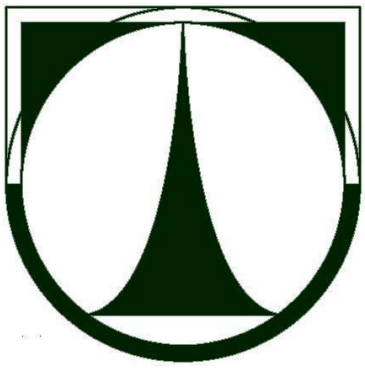

DIPLOMA THESIS

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Technical University of Liberec

Faculty of Textile Engineering

Department of Textile Chemistry

MSc Textile Engineering

DIPLOMA THESIS

Biotechnology in Textile Pre-treatment

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Annotation

Today the use of enzymes in textile processing and after-care is already well established industrial technology. Enzymatic process applications have increased substantially due to developments in genetic engineering, as specific enzymes can be efficiently modified for targeted applications. In addition, being biological molecules and efficient catalysts, enzymes can provide environmentally acceptable route to replace harsh chemicals. Furthermore enzymatic processes can be applied using equipment already existing in the textile industry.

The cellulases of the soft-rot fungus. Trichoderma reesei are the most studied and understood of all cellulolytic systems. Cellulases are used for modification of cellulosic fibres and fabrics, e.g. cotton, viscose and lyocell, yielding properties such as stonewashing, peach-skin and biofinishing effects. Cellulases are usually applied as multi-component enzyme systems and most of the commercial cellulases contain a variety of different activities. The cellulolytic system of T. reesei is composed of two cellobiohydrolases (CBHI and CBHII), at least six endoglucanases and two β-glucosidases. Cellulases are known to act synergistically in the hydrolysis of crystalline cellulose. Endoglucanases randomly attack the amorphous regions in cellulosic substrates, whereas cellobiohydrolases can also act the crystalline regions of cellulose, releasing cellobiose from the ends of cellulose chain. In the present investigation, purified T. reesei cellulases CBHI, CBHII, EGI and EG II were used to treat different types of cotton fabrics in order to evaluate the effects of individual mono-component cellulases on cotton properties. By comparing the impact of mono-component cellulases on cotton twill and poplin
woven fabrics and interlock knitted fabric; it became apparent that cellobiohydrolases and endoglucanases have different effects on the tested fabrics. CBHII did not have any pronounced effect on cotton. By contrast CBHI4 produced significant amounts of reducing sugars and caused weight loss of fabrics. When a high hydrolysis degree was used, i.e. the weight loss was
pronounced, EGII caused more strength loss than either EGI or CBHs. By limiting the treatment time and using additional mechanical action it was observed that EGII was able to improve the pilling properties of cotton fabrics even at low weight and strength loss levels. In addition, the possible synergistic effects between different cellulases were evaluated with different ratios of endo- and exoglucanases. According to weight loss and reducing sugar analyses, both endoglucanases exhibited clear synergism with CBHI. EGI also showed slight synergism with CBHII. Practically no endoendo or exo-exo synergism was observed on the basis of weight loss analysis. Compared to cellulase mixtures, the EGII treatment alone improved the pilling resistance more and resulted in less weight and strength losses at the same protein dosages. Thus, there was no correlation between high weight loss and good pilling results. On the basis of the knowledge obtained from the mono-component treatments, new cellulase preparations with different profiles of T. reesei cellulases were developed. Using these experimental cellulases, it was found that high pilling removal was dependent on the fabric type, and again EGII-based cellulose products yielded the most positive depilling results. It was also shown that the strength loss could be minimized by having only EGII present in the cellulose mixture.

The effects of the purified endoglucanases and cellobiohydrolases and an experimental cellulase mixture on denim were also evaluated. The results confirmed that endoglucanases are the cellulases required for a good stone washing effect, and EGII was the most effective in removing colour from denim despite a very low hydrolysis level. CBHI did not produce any stone washing effect. When the impact of purified cellulases on the molecular weight distribution of cotton powder obtained after enzyme treatment was studied; EGII was the only enzyme which reduced the molecular weight of cotton powder with high mechanical action. The results also showed that mechanical agitation affected the performance of EGII more than that of EGI measured as weight loss and molecular weight of cotton powder.

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1 Introduction

Nowadays, our lives are increasingly changed by the wide application of high and new technologies. Biotechnology is such a technology, which offers the textile industry the ability to reduce costs, protect the environment, address health and safety and improve quality and functionality. Especially as more and more strict laws and regulations on the wastewater discharge were established and implemented, there is a golden opportunity for biotechnology to replacing the traditional textile processing.

Cotton fibers contain approximately 10% of non-cellulosic "impurities" whose contents depend on variety and growing environment. Pectin is one of the main non-cellulosic "impurities" of cotton fiber and it is located mainly in the cuticle of the primary wall. Pectins are a family of complex polysaccharides that contain 1, 4-linked *α*-d-galactosyluronic acid (Gal*p*A) residues. The hydrophobic nature of waxes and pectins is responsible for the non-wetting behavior of native cotton and impedes uniform and efficient dyeing and finishing commonly performed under aqueous conditions.
Current pretreatment processes, using harsh chemicals and severe conditions, are problematic from an environmental point of view because of the high COD, BOD, pH, and salt content in textile effluents and high air pollution due to high energy consumption. On the other hand cellulose is susceptible to oxidation damage under the alkaline treatment conditions, which might result in decreased tensile strength of the fabrics. Alkaline scouring may also cause fabric shrinkage and changes in physico-mechanical properties of the fabric, e.g. their handle.

Starch is widely used as a sizing agent, being readily available, relatively cheap and based on natural, sustainable raw materials. 75% of the sizing agents used worldwide is starch and its derivatives.
Using amylases enzymes for the removal of starch sizes is one of the oldest enzyme applications. Amylases are enzymes which hydrolyze starch molecules to give diverse products, including dextrins and progressively smaller polymers composed of glucose units. These partly degraded oligosaccharides cannot be reused and are usually discharged, contributing large amounts of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) to effluent streams. 50-80% of the COD in the effluents of textile finishing industries is caused by sizing agents.

There are basically four groups of starch-converting enzymes:
(i) Endoamylases
(ii) Exoamylase
(iii) Debranching enzymes
(iv) Transferases

Endoamylases are able to cleave α, 1-4 glycosidic bonds present in the inner part of the amylase or amylopectin chain. α-Amylase is well known endoamylase. The end product of α-amylase action are oligosaccharides. Enzymes belonging to the second group, the exoamylases, either exclusively cleave α, 1-4 glycosidic bonds such as β-amylase or cleave both α, 1-4 and α, 1-6 glycosidic bonds like amyloglucosidase or glucoamylase. Exoamylases act on the external glucose residues of amylase or amylopectin and thus produce only glucose (glucoamylase and α-glucosidase), maltose or dextrin (β-amylase).

The third group of starch-converting enzymes is the debranching ones that exclusively hydrolyze α, 1-6 glycosidic bonds: isoamylase and pullanase.
The fourth group of starch converting enzymes are transferases, which cleave the α, 1-4 glycosidic bond of the donor molecule and the transfer part of the donor to a glycosidic acceptor with the formation of a new glycosidic bond.

An enzymatic process is proposed to utilize desizing baths for bleaching in which glucose oxidase (GOx) enzymes generate hydrogen peroxide and gluconic acid using glucose as a substrate. Advantages of the process are reducing the COD of the effluents by degrading glucose units, and reducing the use of peroxide stabilizing agents with the help of gluconic acid, which is capable of complexing catalysts as well as saving water and energy by using desizing liquor for bleach. However, starch has to be degraded with glucose units in order to achieve process efficiency because of the high substrate selectivity of GOx enzyme.

Conventional commercial desizing enzymes do not seem appropriate for this purpose since most include α-amylase in formulations, whereas amyloglucosidases are suitable amylase enzymes to degrade starch until it becomes glucose.
The performance of an amyloglucosidases / pullanases mixture commercial enzyme used in the food industry to produce glucose syrup from corn starc.

2 Theoretical Part

2.1 Description of enzymes

2.2 Enzymes

Enzymes are proteins that catalyze or increase the rate of chemical reactions. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules, called the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speeds up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell [1].

The activities of enzymes have been recognized for thousands of years, the fermentation of sugar to alcohol by yeast is among the earliest examples of **biotechnological processes.** However, only recently have the properties of enzymes been understood properly. Indeed, research on enzymes has now entered a new phase with the fusion of ideas from protein chemistry, molecular biophysics and molecular biology. Full accounts of the chemistry of enzymes, their structure, kinetics and technological potential can be found in many books and series devoted to these topics. This chapter reviews some aspects of the history of enzymes, their nomenclature, their structure and their relationship to recent developments in molecular biology [3].

Like all catalysts, enzymes work by lowering the activation energy (E_a^+) for a reaction, thus dramatically increasing the rate of reaction.
NB: A catalyst is a substance that initiates or accelerates a chemical reaction without itself being affected whereas an enzyme are mainly proteins that catalyze or increase the rate of chemical reactions, in enzymatic reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different molecules called the products.

Most enzyme reaction rates are millions of time faster than those of comparable un-catalyzed reactions. As with all catalysts, enzymes are not consumed by the reaction they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts by being much more specific. Enzymes are known to catalyze about 4000 biochemical reactions. A few RNA (ribonucleic acid) molecules called ribozymes also catalyze reactions, with an important example being some parts of a ribosome.

Enzyme activity can be affected by other molecules. Inhibitors are the molecules that decrease enzyme activity whereas activators are the molecules that increase activity. Many drugs and poisons are enzyme inhibitors. Activity can also be affected by temperature, chemical environment (e.g. pH) and the concentration of the substrate [2] [3]. Some enzymes are used commercially, for example, the synthesis of anti-biotic. In addition, some household products used enzymes to speed up biochemical reactions e.g. enzymes in biological washing powders breakdown protein or fat stains on clothes, enzymes in meat tenderizers break down proteins-

making the meat easier to chew. Some enzymes are also used in **textile industries** and also used in **waste water treatment.**

In this thesis we will discuss about the ***Biotechnology in pre-treatment of textiles.***

2.3 Etymology (Origin) and History

Early Concepts of Enzymes

The term ''enzyme'' was coined by **Kuhne** in 1876. Yeast, because of the acknowledged importance of fermentation, was a popular subject of research. A major controversy at that time, associated most memorably with Liebeg and Pasteur, was whether or not; the process of fermentation was separable from the living cells.

No belief in the necessity of vital forces, however, survived the demonstration by Eduard Buchner (1897) that alcoholic fermentation could be carried out by a cell-free yeast extract. The existence of extra-cellular enzymes had, for reasons of experimental accessibility, already been recognized. For example as early as 1783, Spallanzani had demonstrated that gastric juice could digest meat in vitro and Schwann (1836) called the active substance pepsin. Kuhne himself appears to have given trypsin its present name, although its existence in the intestines had been suspected since the early 1800s [3].

Having shown that enzymes could function outside living cell, the next step was to determine their bio-chemical nature. Many early workers noted that enzymatic activity was associated with proteins but several scientists such as Nobel laureate Richard Willstatter argued that proteins were merely carriers for the true enzymes and the proteins were incapable of catalysis.

However, in 1926, James B. Sumner showed that enzyme urease was a pure protein and crystallized it. Sumner did likewise for the enzyme catalase in 1937. The conclusion that pure proteins can be enzymes was definitely proved by Northrop and Stanley, who worked on the digestive enzymes pepsin (1930), trypsin and chymotrypsin. These three scientists were awarded the 1946 Nobel Prize in Chemistry.

The discovery that enzymes could be crystallized eventually allowed their structures to be solved by x-ray crystallography. This was first done for lysozyme, an enzyme found in tears, saliva and egg whites that digest the coating of some bacteria, the structure was solved by a group led by David Chilton Phillips and published in 1956. This high-resolution structure of lysozyme marked the beginning of the field of structural biology and the effort to understand how enzymes work at an atomic level of detail [1].

2.4 Structures and Mechanisms

2.4.1 Mechanisms

The enzymes contain true activity centers in the form of three-dimensional structures as fissures, holes, pockets and cavities or hollows. The active site is a part of the enzyme molecule that combines with the substrate. The number of active sites per enzyme molecule is very small, generally only one. To catalyze the reaction, the enzyme molecule makes a complex adsorbed onto the surface of substrate in lock and key fashion. This lock and key template model (Figure 1) is still useful for understanding certain properties of enzymes. We can show the action of enzyme as follows:

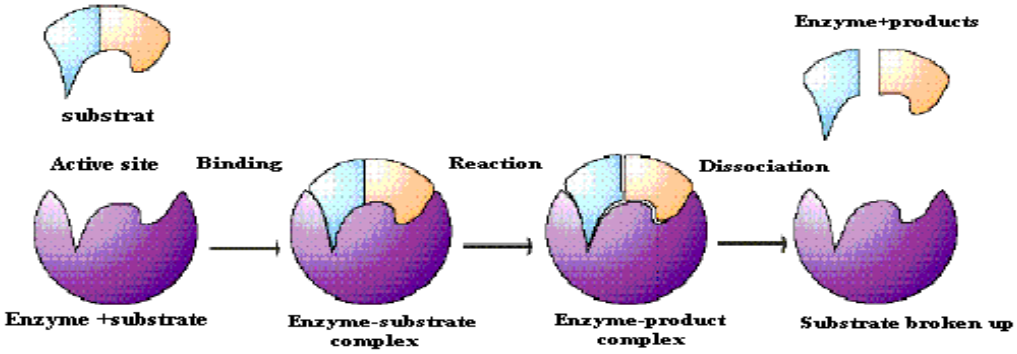


Fig 1: Enzyme action is often explained by the analogy of the lock and key. The substrate must fit portion of the enzyme called active site. This process also weakens the bonds that holds the substrate and fragments it. The substrate is then easily rinsed away.

2.4.2 ''Lock and Key'' model

Enzymes are very specific, and it was suggested by the Nobel laureate organic chemist Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as ''lock and key'' model. However, while this model explains enzyme specificity, it fails to explain stabilization of the transition state that enzymes achieve.

In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continually re-shaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply

bind to a rigid active site; the amino side chains which make up active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidase, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge are determined. Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via conformational proof-reading mechanism [8].

2.4.3 How Enzymes Work?

For two molecules to react they must collide with one another. They must collide in the right direction (orientation) and with sufficient energy. Sufficient energy means that between them, they have enough energy to overcome the energy barrier to reaction. This is called the *Activation Energy.*

2.4.4 Reaction Profile

Enzymes have an *active site*. This is the part of the molecule that has just the right shape and functional groups to bind to one of the reacting molecules. The reacting molecule that binds to the enzyme is called the *substrate*. An enzyme-catalyzed reaction takes a different 'route'. The enzyme and substrate form a reaction intermediate. Its formation has lower activation energy than the reaction between reactants without a catalyst.

A simplified picture

Route a reactant 1 + reactant 2 → product

Route B reactant 1 + enzyme → intermediate

Intermediate + reactant 2 → product + enzyme

So the enzymes are used to form a reaction intermediate, but when this reacts with another reactant the enzyme reforms.

2.5 Co-enzymes and Co-factors

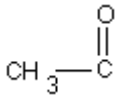
2.5.1 Co-enzymes

Co-enzymes are organic molecules that are required by certain enzymes to carry out catalysis. They bind to the active site of the enzyme and participate in catalysis but are not considered substrates of the reaction [9]. Some of these chemicals such as riboflavin, thiamine and folic acids are vitamins (compounds which cannot be synthesized by the body and must be acquired from the diet). The chemical groups carries include the hybrid ion (H-) carried by NAD or NADP+, the phosphate group carried by adenosine triphosphate, the acetyl group carried by co-enzyme A, formly, methenyl or methyl groups carried by folic acid and the methyl group carried by S-adenosyl methionine.

Since co-enzymes are chemically changed as a consequence of enzyme action, it is useful to consider co-enzymes to be a special class of substrates or second substrates which are common to many different enzymes. For example, about 700 enzymes are known to use the co-enzyme NADH [1].

Some coenzymes and the reactions they are involved in are shown in the following table:

TABLE 1: CO-ENZYMES IN GROUP TRANSFER REACTIONS

Co-enzyme	Abbreviation	Entity transfered
nicotine adenine dinucelotide	NAD - partly composed of niacin	electron (hydrogen atom)
nicotine adenine dinucelotide phosphate	NADP -Partly composed of niacin	electron (hydrogen atom)
flavine adenine dinucelotide	FAD - Partly composed of riboflavin (vit. B2)	electron (hydrogen atom)
coenzyme A	CoA	Acyl groups 
coenzymeQ	CoQ	electrons (hydrogen atom)
thiamine pyrophosphate	Thiamine (vit. B1)	aldehydes
pyridoxal phosphate	pyridoxine (vit B6)	amino groups
biotin	biotin	carbon dioxide
carbamide coenzymes	Vit. B12	alkyl groups

2.5.2 Co-factors

Some enzymes do not need any additional components to show full activity. However, others require non-protein molecules called co-factors to be bound for activity. Co-factors can be either inorganic e.g. metal ions or iron sulfur clusters, or organic compounds e.g. flavin and heme.
Organic co-factors can be either prosthetic group, which are tightly bound to an enzyme or co-enzymes, which are released from the enzyme's active site during the reaction. Co-enzymes include NADH, NADPH and adenosine triphosphate. These molecules transfer chemical groups between enzymes [1] [9].

Examples of some enzymes that require metal ions as co-factors are shown in the table below:

TABLE 2: CO-FACTORS AND ENZYMES

Co-factor	Enzyme or Protein
Zn++	carbonic anhydrase
Zn++	alcohol dehydrogenase
Fe+++ or Fe++	cytochromes, hemoglobin
Fe+++ or Fe++	ferredoxin
Cu++ or Cu+	cytochrome oxidase
K+ and Mg++	pyruvate phosphokinase

2.6 Thermodynamics

As all catalysts, enzymes do not change the position of the chemical equilibrium of the reaction. Usually, in the presence of an enzyme, the reaction runs in the same direction as it would without enzyme, just more quickly. However, in the absence of the enzyme, other possible un-catalyzed, 'spontaneous' reactions might lead to different products, because in those conditions this different product is formed faster [2].

Furthermore, enzymes can couple two or more reactions, so that a thermodynamically favorable reaction can be used to 'drive' a thermodynamically unfavorable one. For example, the hydrolysis of ATP is often used to drive other chemical reactions.
Enzymes catalyze the forward and backward reactions equally. They do not alter the equilibrium itself, but only the speed at which it is reached. For example, carbonic anhydrase catalyzes its reaction in either direction depending on the concentration of its reactants.

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$ (In tissues; high CO_2 concentration)

$$H_2CO_3 \xrightarrow{\text{Carbonic anhydrase}} CO_2 + H_2O$$ (In lungs; low CO_2 concentration)

Nevertheless, if the equilibrium is greatly displaced in one direction, that is, in a very exorgenic reaction, the reaction is effectively irreversible. Under these conditions the enzyme will, in fact, only catalyze the reaction in the thermodynamically allowed direction [1] [2].

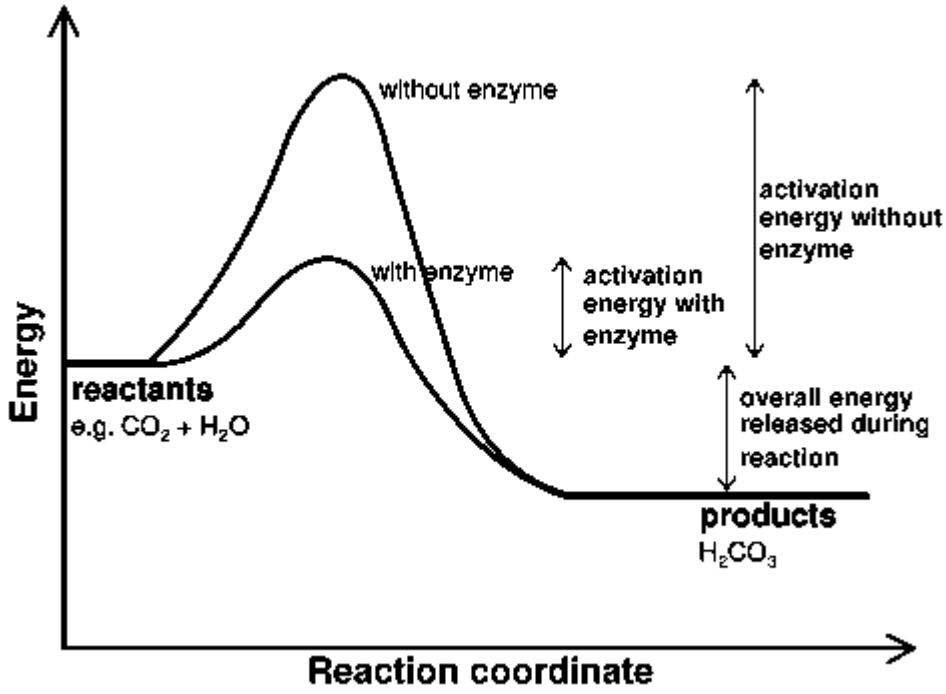


Fig 2: The energy of the stages of a chemical reaction

Substrates need a lot of energy to reach a transition state, which then decays into products. The enzyme stabilizes the transition state, reducing the energy needed to form products.

There are two general ways of increasing the rate of a chemical reaction. One is to increase the reaction temperature in order to increase the thermal motion of the molecules and thus increase the fraction having sufficient energy internal energy to enter the transition state.

The second way of accelerating a chemical reaction is to add a catalyst, e.g., an enzyme. Catalysts enhance reaction rates by lowering activation energies. In enzymatic reactions, binding groups and catalytic centers (active sites) in enzyme molecules bind substrate molecules to form intermediate complexes with lower energy contents than those of the transition states of the un-catalyzed reactions. These complexes undergo certain atomic and electronic rearrangements, after which the products are released, see figure 1. Thus, the enzymes work by providing

alternative reaction pathways with lower activation energies than those of the un-catalyzed reaction, see figure 2 [10].

2.7 Inhibition

Enzyme reaction rate can be decreased by various types of enzyme inhibitors. These are molecules that bind to enzymes and decrease their activity. Not all molecules that bind to enzymes are inhibitors. The enzyme activators bind to enzymes and increase their enzymatic activity. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically [7].

2.7.1 Inhibition of enzyme activity

Some substances reduce or even stop the catalytic activity of enzymes in bio-chemical reactions. They block or distort the active site. These chemicals are called *inhibitors,* because they inhibit the reaction.

Inhibitors that occupy the active site and prevent the substrate molecule from binding to the enzyme are said to be active site-directed or competitive as they 'compete' with the substrate for the active site.

Inhibitors that attach to other parts of the enzyme molecule, perhaps distorting its shape, are said to be non-active site-directed or non-competitive.

2.7.2 Immobilized Enzymes

Enzymes are widely used commercially, for example in the detergent, food and brewing industries. Protease enzymes are used in 'biological' washing powders to speed up the breakdown of proteins in stains like blood and egg. Pectinase is used to produce and clarify fruit juices. Problems using enzymes commercially includes [14]:

1. They are water soluble which makes them hard to recover.
2. Some products can inhibit the enzyme activity (feedback inhibition)

Enzymes can be immobilized by fixing them to a solid surface. This has a number of commercial advantages:

- the enzyme is easily removed
- the enzyme can be packed into columns and used over a long period
- speedy separation of products reduces feedback inhibition
- thermal stability is increased allowing higher temperatures to be used
- higher operating temperatures increase rate of reaction

There are four principal methods of immobilization currently in use:

- covalent bonding to a solid support
- adsorption onto an insoluble substance
- entrapment within a gel
- encapsulation behind a selectively permeable membrane

2.7.3 Competitive inhibition

In competitive inhibition, the inhibitor and substrate compete for the enzyme (i.e., they cannot bind at the same time). Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, methotrexate (fig 4) is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate. The similarity between the structures of folic acid and this drug are shown in figure 4. Note that binding of the inhibitor need *not* be to the substrate binding site, if binding of the inhibitor changes the conformation of the enzyme to prevent substrate binding and *vice versa*. In competitive inhibition the maximal velocity of the reaction is not changed, but higher substrate concentrations are required to reach a given velocity, increasing the apparent K_m.

2.7.4 Uncompetitive inhibition

In uncompetitive inhibition the inhibitor cannot bind to the free enzyme, but only to the ES-complex. The EIS-complex thus formed is enzymatically inactive. This type of inhibition is rare, but may occur in multimeric enzymes as shown in figure 3.

2.7.5 Non-competitive inhibition

Non-competitive inhibitors can bind to the enzyme at the same time as the substrate, i.e. they *never* bind to the active site. Both the EI and EIS complexes are enzymatically inactive, because the inhibitor cannot be driven from the enzyme by higher substrate concentration (in contrast to competitive inhibition), the apparent V_{max} changes. But because the substrate can still bind to the enzyme, the K_m stays the same [7].

2.7.6 Mixed inhibition

This type of inhibition resembles the non-competitive, except that the EIS-complex has residual enzymatic activity. In many organisms inhibitors may act as part of a feedback mechanism. If an enzyme produces too much of one substance in the organism, that substance may act as an inhibitor for the enzyme at the beginning of the pathway that produces it, causing production of the substance to slow down or stop when there is sufficient amount. This is a form of negative feedback. Enzymes which are subject to this form of regulation are often multimeric and have allosteric binding sites for regulatory substances. Their substrate/velocity plots are not hyperbola, but sigmoidal (S-shaped).

Irreversible inhibitors react with the enzyme and form a covalent adduct with the protein. The inactivation is irreversible. These compounds include eflornithine a drug used to treat the parasitic disease sleeping sickness. Penicillin and Aspirin also act in this manner. With these drugs, the compound is bound in the active site and the enzyme then converts the inhibitor into an activated form that reacts irreversibly with one or more amino acid residues [7].

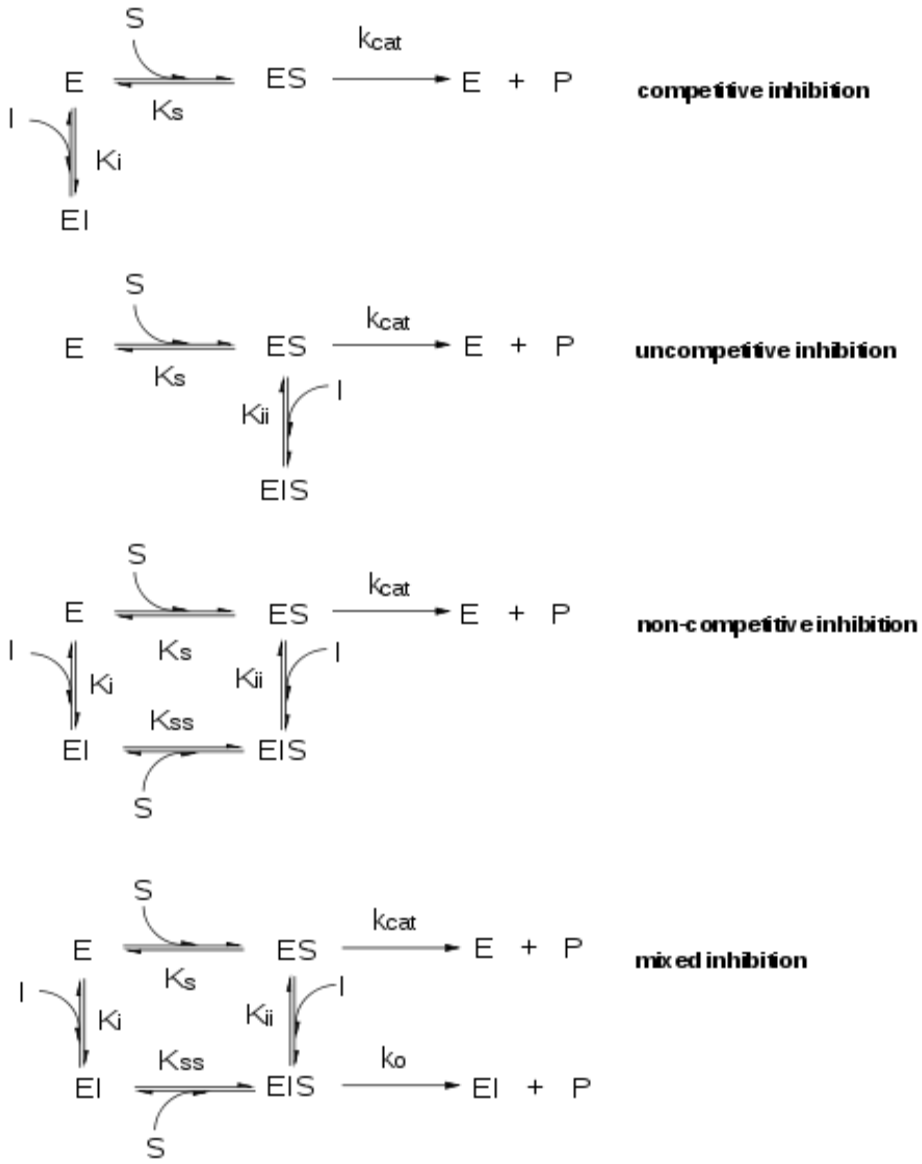


Fig 3: Types of inhibition

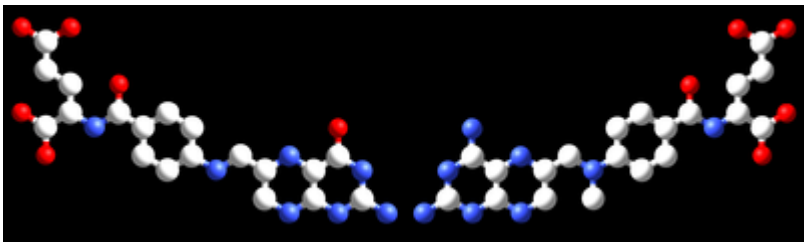


Fig 4: The coenzyme folic acid (left) and the anti-cancer drug methotrexate (right) are very similar in structure. As a result, methotrexate is a competitive inhibitor of many enzymes that use foliates [1] [2].

2.7.7 Uses of inhibitors

Since inhibitors modulate the function of enzymes they are often used as drugs. An common example of an inhibitor that is used as a drug is an aspirin, which inhibits the COX-1 and COX-2 enzymes that produce the inflammation messenger prostaglandin, thus suppressing pain and inflammation. However, other enzyme inhibitors are poisons. For example, the poison cyanide is an irreversible enzyme inhibitor that combines with the copper and iron in the active site of the enzyme cytochrome oxidase and blocks cellular respiration [14].

2.8 Properties of Enzymes

(a) Enzymes accelerate reactions

Enzymes speed up a particular chemical reaction by lowering the activation energy for the reaction. They achieve this by forming an intermediate enzyme-substrate complex, which alters the energy of the substrates such that it can be more readily converted into the product. The enzyme itself is unaltered at the end of the reaction, thus acting as a catalyst. Enzymes have an amazing catalytic power. They accelerate the reaction which is often undetectable in the absence of enzyme by enormous amounts, sometimes several million folds.

(b) Enzymes act only on specific substrates

One of the most important properties of enzymes is its specific gravity, which describes the enzymatic strength towards a particular substrate. Most enzymes have a high degree of specificity and will catalyze a reaction with only one or a few substrates. There are exceptions and some proteases have a fairly low specificity to protein substrates. However, one particular enzyme will only catalyze a specific type of reaction. There are six classes of enzyme [7]:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Layases
5. Isomerases and
6. Ligases

Most of the enzymes used in the textile industries belong to *Hydrolases*. Hydrolases catalyze reactions in the form of Equ (1).

$$A\text{-}X + H_2O \rightarrow X\text{-}OH + HA \quad \text{.....(1)}$$

(c) Enzymes operate under mild conditions

Most enzymes have a maximum activity at an optimum temperature, which is often the temperature within the cell media from which the enzyme was derived. For extra cellular enzyme of a particular organism or selected by a microorganism, the optimum temperature may be that of the environment in which the enzyme normally operates. The reaction rate increases with the

increasing temperature until the optimum temperature is reached. Above this value enzyme decreases rapidly until a point where enzymes become permanently deactivated by denaturation. Temperatures below the optimum range decrease the enzyme activity without damaging the protein structure. Lower ranges of temperature can be used with longer times of exposure to the substrate. Enzymes also have an optimum pH, and its activity decreases sharply on both sides of the optimum range.

(d) Enzymes are easy to control

As they are protein molecules, enzymes are potential antigens to humans and therefore elicit on immunological response. Exposure to enzymes in droplet form must be avoided, however, providing precautions are taken to avoid spills, ingestion or prolonged skin exposure to concentrated enzymes power. The use of enzymes in industries is as safe as, and in most instances safer than using any of other chemicals employed in the textile industry.

Enzymes are easy to control, they will perform well at the optimum temperature and pH but when they are no longer required, they can be deactivated by altering the temperature or pH levels [7].

(e) Enzymes can replace harsh chemicals

The use of enzyme can often replace a chemical reaction that, if not rigidly controlled is harmful to the substrate under treatment. The chemical employed may also be toxic or harmful to man and are better avoided where possible.

(f) Enzymes are biodegradable

When the enzyme has catalyzed the required reaction and is no longer needed, altering temperature and or pH may inactive it. Such conditions generally result in an irreversible change in protein structure. Enzymes will also be inactivated in bleaching reactions. Extremely severe chemical conditions are necessary to catalyze the enzyme protein (e.g. 6 M Hcl @ 100^0C for 24hr) to its amino acid components.

2.9 Catalytic Activities of Enzymes

Many enzyme reactions may be modeled by the reaction scheme:

$$E + S \leftrightarrow ES \rightarrow E + P \quad \text{.....(2)}$$

Where E, S and P represent the enzyme, substrate and product, respectively and ES represent an *enzyme-substrate complex as shown in equ. (2)* . Usually, it is assumed that the equilibrium between S and ES is established rapidly, so that the second reaction is the one mainly determining the rate d [P]/dt of appearance of the product P.

This reaction will follow a first order rate law, i.e.

$$d[P]/dt = -k_{cat}[ES] \quad \text{.....(3)}$$

With a rate constant k_{cat} called *catalytic constant* or the *turnover number* [10]. Under commonly used conditions of enzyme activity measurement, k_{cat} can be considered to be sufficiently constant during the observed reaction period (steady-state assumption, BRIGGS and HALDANE) [3]. Under given conditions and at given initial concentrations [E] and [S] of enzyme and substrate, respectively, the rates of appearance of P will typically decrease overtime. The rate observed during conversion of the first few percent of the substrate is called the initial rate *V*. In 1913, Leonor Michaelis and Maud Menten showed that the above model leads to the following relation between the initial rate *V* and the initial substrate enzyme concentration [S] at any given enzyme concentration:

$$V = \frac{V_{max}[S]}{K_M + [S]} \quad \text{.....(4)}$$

Where K_M is a constant called now *Michaelis constant* and V_{max} is a constant dependent on the enzyme concentration. This dependence of *V* on [S] leads to the characteristic curve shape. At low substrate concentrations, the initial rate is, with good approximation, proportional to [S], and at high values of [S] (substrate saturation) it approaches the limit value $V_{max,}$ aptly called the maximum rate.

The calculations further show that $V_{max} = K_{cat}[E]$ .....(5)

The Michaelis constant is independent of the enzyme concentration, and it can be seen from the formula above that K_M can be found as the substrate concentration for which $V = V_{max}/2$, equ (5).
In general, for given enzyme, different substrates and different sets of conditions (temperature, pH) will give different values of k_{cat} and K_M and thus different initial rates will be measured under otherwise identical conditions. This means in practice that each enzyme has an optimum range of pH and temperature for its activity with a given substrate. The presence or absence of *co-factors* and *inhibitors* may also influence the observed kinetics.
Enzyme activity is usually determined using a rate assay and expressed in activity units. The substrate **concentration**, **pH** and **temperature** are kept constant during these assay procedures. Standardized assay methods are used for commercial enzyme preparations [10].

2.9.1 Factors Governing Catalytic Activities

2.9.1.1 Temperature

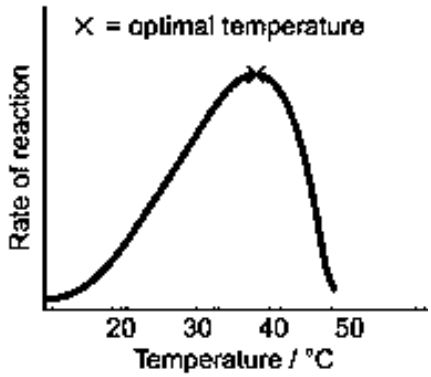


Fig 5: As the temperature rises, reacting molecules have more and more kinetic energy

As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a cetain temperature at which an enzyme's catalytic is at its greatest, see figure 5. This optimal temperature is usually around human body temperature (37.5 ^{0}C) for enzymes in human cells.
Above this temperature, the enzyme structure begins to break down (denature) since at higher temperatures intra and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy. Some temperature – insensitive enzymes may exhibit an optimum at almost 100^{0}C for example, pepsin, saccharase and trypsin [11] [3].

2.9.1.2 pH

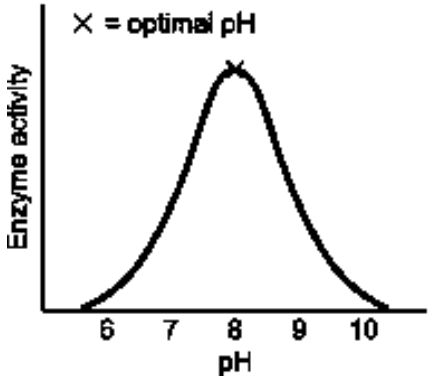


Fig 6: The enzymes works within a quite small pH range

Each enzyme works within a quite small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra and intermolecular bonds, changing the shape of and enzyme and, therefore, its effectiveness.
The optimum depends not only on pH but also on ionic strength and type of buffer. It may also be influence by temperature, substrate and coenzyme concentrations. For most enzymes, the pH optimum lies in the range from 5 to 7. Extreme values of 1.5 and 10.5 have been found for pepsin and for alkaline phosphatase respectively [11][13].

2.9.1.3 Concentration of enzyme and substrate

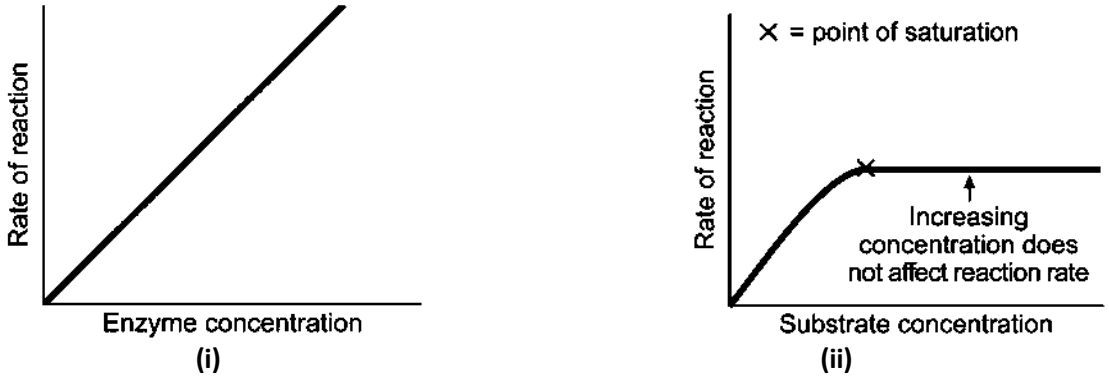


Fig 7: (i) As the concentration of either increased, the rate of reaction increases, (ii) For a given enzyme concentration, the rate of reaction increasing with increasing substrate concentration

The rate of an enzyme-catalyzed reaction depends on the concentrations of enzyme and substrate. As the concentration of either increased, the rate of reaction increases (see figure 7).
For a given enzyme concentration, the rate of reaction increasing with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate (see graphs).
Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration (see graph).

2.9.1.4 Activation

Many chemical effectors activate or inhibit the catalytic activity of enzymes. In addition to substrates and coenzymes, many enzymes require nonprotein or in some cases, protein compounds to be fully active. Enzyme activation by many inorganic ions has been adequately described. The activating ion may be involved directly in the reaction by complexing the coenzyme or cosubstrate (e.g., Fe ions bound to flavin or the ATP-Mg complex). In other cases,

the ion is the part of the enzyme and either acts as a stabilizer for the active conformation (e.g., Zn ions in alkaline phophotase) or participates directly at the active site e.g. Mn ions in isocitrate dehydrogenase and Zn or Co ions in carboxypeptidases [13].

2.9.1.5 Allosteric Modulation

Allosteris sites are sites on the enzyme that bind to molecules in the cellular environment. The sites form weak, noncovalent bonds with these molecules, causing a change in the conformation of the enzyme. This change in conformation translates to the active site, which then affects the reaction rate of the enzyme. Allosteric interactions can both inhibit and active enzymes and are a common way that enzymes are controlled in the body as shown in fig 8.

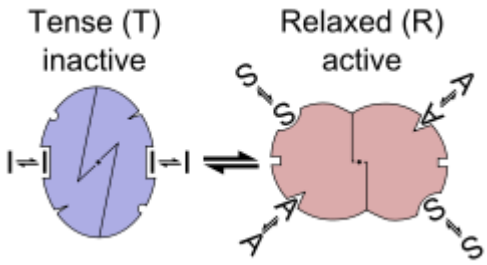


Fig 8: Allosteric transition of an enzyme between R and T states, stabilized by an agonist, an inhibitor and a substrate (the MWC model)

2.10 General Production Methods

2.10.1 Organism and Enzyme Synthesis:

A variety of different microorganisms are used for the industrial production of enzymes. For most of the history of enzyme applications, production occurred in the strain known to make the enzyme of interest. This explains why so many different types of microorganisms have been employed to make enzymes.

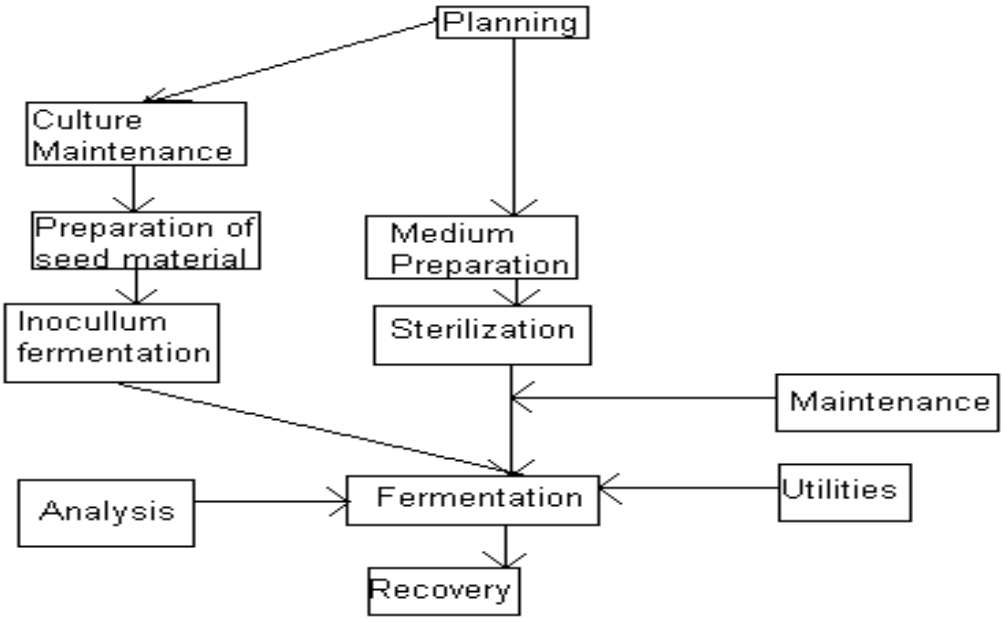


Fig 9: Process of Enzyme Production

Alkaline protease is naturally secreted by *Bacillus licheniformis* to break down proteinaceous substrates and resulted in one of the first commercially produced enzymes. Strains were selected to produce higher levels of protease and an industry was born. A similar story was followed for α – amylase production. Again, *Bacillus licheniformis* naturally secreted a highly thermostable α – amylase capable of breaking down starch to more easily digestible oligosaccharides. Strains of *Bacillus* have been one of the workhorses of enzyme production for decades, based mainly upon their ability to overproduce substilisin and α – amylase.

Amylase from *Bacillus* has historically been used to liquefy starch. However, to break down starch completely into single units of glucose, a second enzyme, namely, gluco-amylase, is required. The most widely used enzyme for glucose production from starch is the gluco-amylase from strains of the fungal genus *Aspergillus.*

An acid cellulase enzyme complex is found in the fungus *Trichoderma.* This enzyme mixture was thought to be capable of breaking down cellulosic substrates to glucose, similar to starch-degrading enzymes. This particular application was not initially commercialized. Instead, it has found application in the **treatment of textiles.** New programs to improve the enzyme complex and its expression are resurrecting its potential use as an additive for the breakdown of cellulose [3].

2.10.2 Process of Enzyme Production

Industrial enzymes are produced using a process called submerged fermentation. This involves growing carefully selected microorganisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen (aerobic conditions). As the microorganisms break down the nutrients, they produce the desired enzymes. Most often, the enzymes are secreted into the fermentation medium as shown in figure 8.
Thanks to the development of large-scale fermentation technologies, today the production of microbial enzymes accounts foe a significant proportion of the biotechnology industry's total output. Fermentation takes place in large vessels called fermentors with volumes of up to 1,000 cubic meters.
The fermentation media comprise nutrients based on renewable raw materials like corn, starch and soy grits. Various inorganic salts are also added depending on the microorganism being grown.
Both fed-batch and continuous fermentation processes are common. In the fed-batch process, sterilized nutrients are added to the fermentor during the growth of the biomass. In the continuous process, sterilized liquid nutrients are fed into the fermentor at the same flow rate as the fermentation broth leaving the system, thereby achieving steady-state production. Operational parameters like temperature, pH, feed rate, oxygen consumption and carbon dioxide formation are usually measured and carefully controlled to optimize the fermentation process [3] [10].

2.11 Enzymes in Textile Industry

2.11.1 Introduction

Nowadays, our lives are increasingly changed by the wide application of high and new technologies. Biotechnology is such a technology which offers the textile industry the ability to reduce costs, protect the environment, address health and safety and improve quality and

functionality. Especially as more and more strict laws and regulations on the waste water discharge were established and implemented, there is a golden opportunity for biotechnology to replace the traditional textile processing.
In 2001, China had popularized some cleaner production techniques including bio-stone-washing of denim with ***cellulases*** and desizing of cotton fabrics with ***amylases*** to country wide enterprises under the policy of prevention and control of textile effluent pollution, the general plan and development of China's textile industry as well as the guide to the cleaner production of textile industry. There are 28 research priorities in the guideline of the 11th Five-Year (2006-2010) science and technology development program for textile industry, and among them, textile biotechnology such as environmentally friendly enzymatic treatments is included.

Cotton fibers contain approximately 10% of non-cellulosic ''impurities'' whose contents depend on variety and growing environment. Pectin is one of the main non-cellulosic ''impurities'' of cotton fibers and is located mainly in the cuticle of the primary wall. Pectins are a family of complex polysaccharides that contain 1, 4-linked α-d-galactosyluronic acid (Gal*p*A) residues.

Current pre-treatment processes, using harsh chemicals and severe conditions are problematic from an environmental point of view because of the high COD, BOD, pH and salt content in the textile effluents and high air pollution due to high energy consumption. On the other hand cellulose is susceptible to oxidation damage under the alkaline conditions, which might result in decreased tensile strength of the fabrics. Alkaline scouring may also cause fabric shrinkage and change in physico-mechanical properties of the fabric e.g. their handle.

The following types of enzymes based on the composition of non-cellulosic ''impurities'' of cotton have been evaluated:

2. Pectinases
3. Amylases
4. Cellulases
5. Xylanases
6. Proteases
7. Lipases
8. Hemicellulases
9. Laccases
10. Catalases

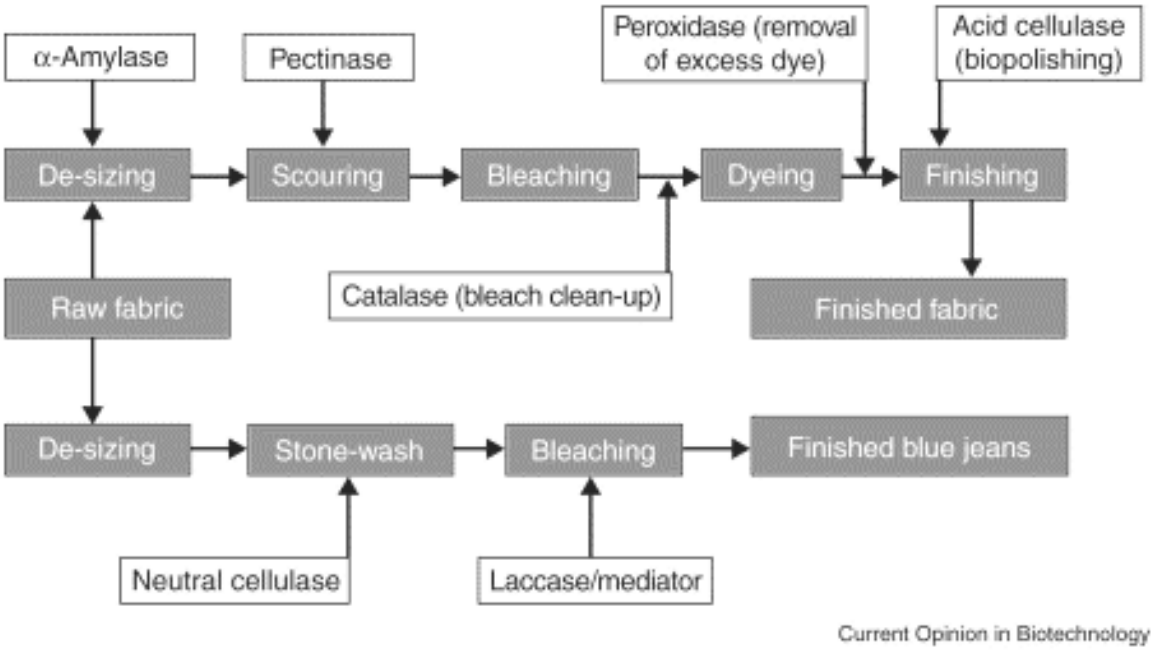


Fig 10: Enzymes that are used in Textile Industry

2.11.2 Cellulases

Cellulase refers to a family of enzymes which act in concert to hydrolyze cellulose. Cellulases are widely distributed throughout the biosphere and are most manifest in fungal and microbial organisms. Cellulase is an enzyme complex which breaks down cellulose to beta-glucose (α – glucose). It is produced mainly by symbiotic bacteria in the ruminating chambers of herbivores. Aside from ruminants, most animals (including humans) do not produce cellulase, and are therefore unable to use most of the energy contained in plant material [12].

Biological degradation of the three constitutes requires many different enzymes to work together a consortium, but most needed enzymes are those which tackle cellulose and hemicelluloses. For complete hydrolysis of cellulose and hemicelluloses, enzymes required are:

- Cellobiohydrolases
- Endoglucanases
- Beta-glucosidases
- Xylanases
- Beta-xylosidases
- Alpha-arabinofuranosidases
- Phenolic and
- Acetyl xylan esterases
- Alpha-glucuronidases and
- Alpha-galactosidases [12].

TABLE 3: CELLULASES AND APPLICATION CONDITIONS - SOURCE: NOVOZYMES CELLULASE APPLICATION SHEETS

Product Name	Micro-organisms	Cellulase Type(s)	pH range	Temperature range
Celluzyme	Thermomyces lanuginosus	CBH I, CBH II, EG I, EG II, EG III, EG VI & EG V	4 – 10	25 – 70
Carezyme	Thermomyces lanuginosus	EG V	5 – 10.5	25 – 70
Endolase	Thermomyces lanuginosus	EG II	5 - 9	25 - 70

2.11.2.1 Types and Action

Five general types of cellulases based on the type of reaction catalyzed:

- Endo-cellulase breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains.
- Exo-cellulase cleaves 2-4 units from the ends of the exposed chains produced by endocellulase, resulting in the tetrasaccharide or disaccharide such as cellobiose. There are two types of exo-cellulases – one type work processively from the reducing end and one type works processively from the non-reducing end of cellulose.
- Cellobiase hydrolysis the exo-cellulase product into individual monosaccharides.
- Oxidative cellulases that depolymerize cellulose by radical reactions, as for instance celloboise dehydrogenase (acceptor).
- Cellulose phosphorylases that depolymerize cellulose using phosphates instead of water.

In the most familiar case of cellulase activity, the enzyme complex breaks down cellulose to beta-glucose.

2.11.2.2 Mechanism of Cellulolysis

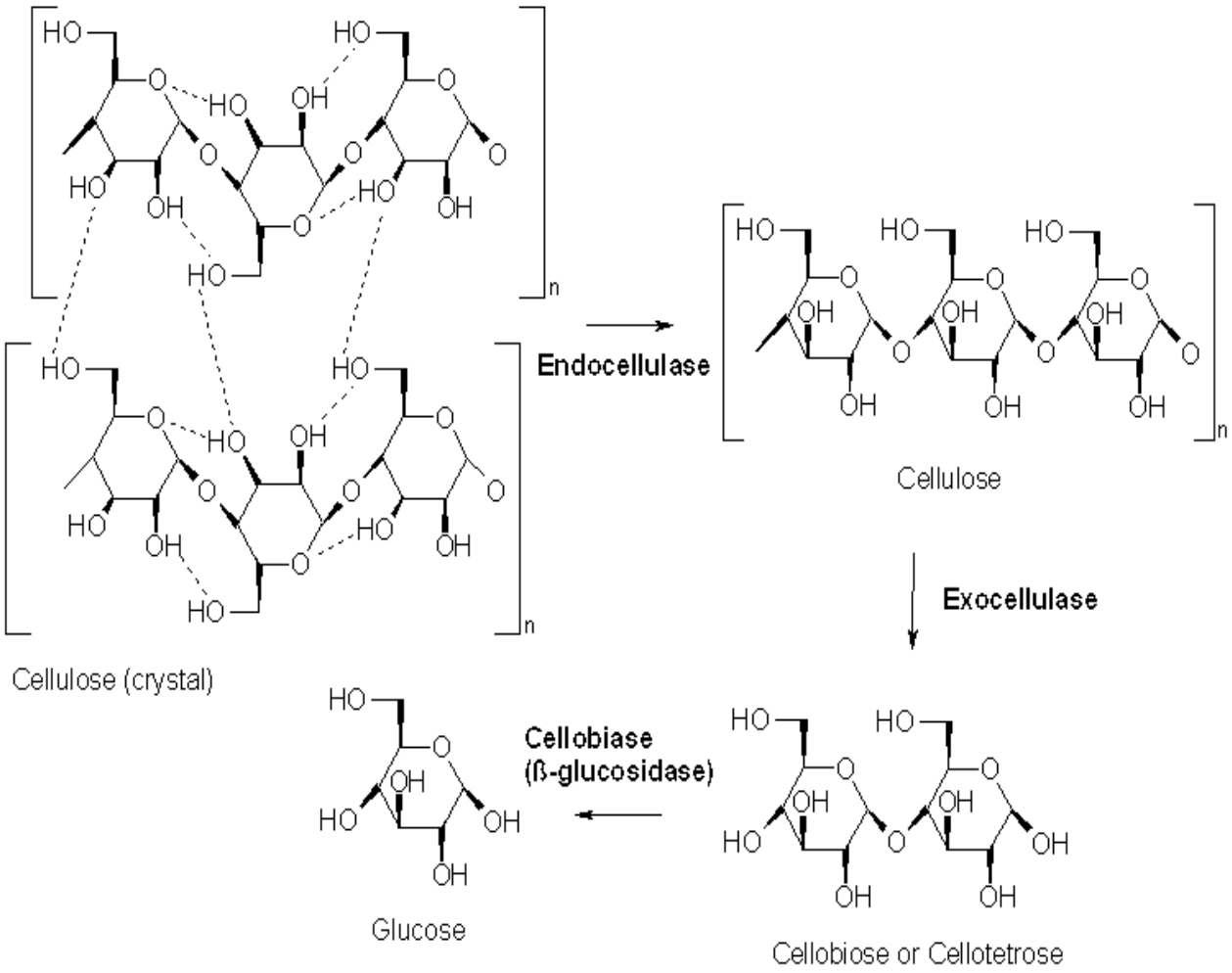


Fig 11: Mechanism of Cellulolysis

The three types of reaction catalyzed by cellulases:

1. Breakage of the non – covalent interactions present in the crystalline structure of cellulose (endo-cellulose).
2. Hydrolysis of the individual cellulose fibers to break them into smaller sugars (exo-cellulase)
3. Hydrolysis of disaccharides and tetrasaccharides into glucose (beta-glucosidase) [13].

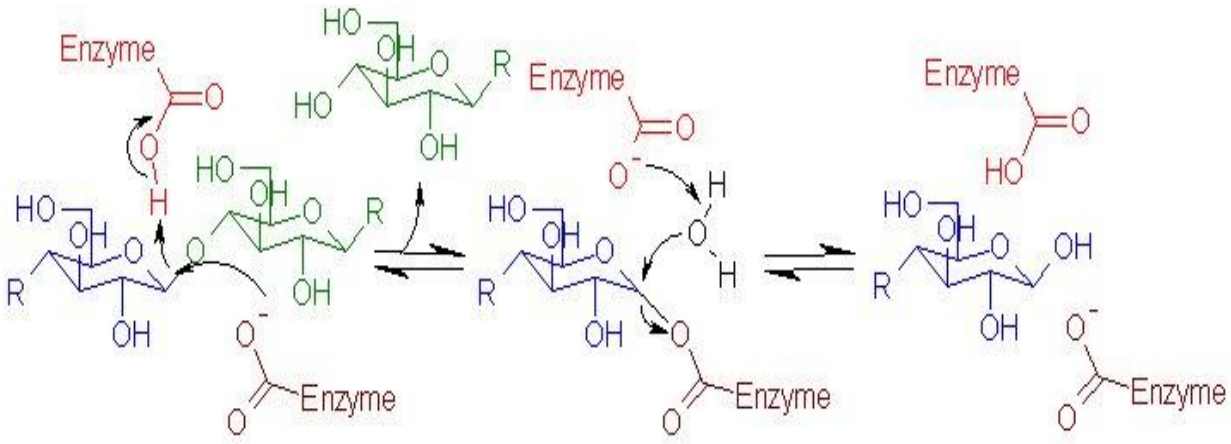


Fig 12: Mechanistic details of beta glucosidase activity of cellulase

2.11.2.3 Cellulases in textile and laundry biotechnology

Cellulases have achieved their world wide success in textile and laundry because of their ability to modify cellulosic fibers in the controlled and desired manner, so as to improve the quality of fabrics. Although, cellulases were introduced in textile and laundry's decade ago, they have now become the third largest group of enzymes used in these applications. ***Bio-stoning and bio-polishing are the best known current textile applications of cellulases*** as shown in table 4. Cellulases are also increasingly used in the household washing powders, since they enhance the detergent performance and allow the removal of small, fuzzy fibrils from fabric surfaces and improve the appearance and color brightness.

TABLE 4: CELLULASES IN TEXTILE AND LAUNDRY BIOTECHNOLOGY

Enzyme	Function	Application
Cellulase, preferably neutral and endoglucanase rich	Removal of excess dye from denim fabrics; soften the cotton fabrics without damaging the fiber	Bio-stoning of fabrics; production of high quality and environmentally friendly washing powders
Cellulase, preferably acid and endoglucanase rich	Removal of excess micro fibrils from the surface of cotton and non-denim fabrics	Bio-polishing of cotton and non-denim fabrics
Cellulase, preferably endoglucanase rich	Restoration of softness and color brightness of cotton fabrics	Production of high quality fabrics

2.11.2.4 Specifications of cellulases are shown in the table below

TABLE 5: SPECIFICATIONS OF CELLULASES

Enzyme	Cellulase	Neutral Cellulase	Cellulase
Activity	2.200 (FPA)	1.100 (DCMC)	9.500 (WCMC)
Application Temperature (^{0}C)	45-55	45-55	45-55
Application pH	5.0-6.0	6.5-7.5	5.0-6.0
Commercial Equivalent	Novoprime 939 (Novo)	Cellusoft CR (Novo)	Novoprime 959 (Novo)

2.11.2.5 Safety

Inhalation of the enzyme mists should be avoided. In case of contact with the skin and mucous membranes such as eyes, mouth etc., rinse thoroughly with water. In case of irritation or allergic symptoms, seek medical attention. The details make reference to MSDS.

2.11.3 Amylases

2.11.3.1 Definition of Amylases

Amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva, where it begins the chemical process of digestion. The pancrease also makes amylase (alpha amylase) to hydrolyze dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply body with energy. Plants and some bacteria also produce amylase. Amylase was the first enzyme to be discovered and isolated by Anselme and Payen in 1833. Specific amylase proteins are designated by different Greek letters. All amylases are glycoside hydrolases and act on α-1.4 glycosidic bonds [15].

2.11.3.2 Classification of Amylases

α - Amylase
(EC 3.2.1.1) (CAS # 9014-71-5) (Alternate names: 1.4-α-D-glucan glucanohydrolase; glycogenase)

The – amylase are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotrriose and maltose from amylose or maltose or glucose and ''limit dextrin'' from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster acting than β-amylase. In animals, it is a major digestive enzyme and its optimum pH is 6.7-7.0 [15].

β - Amylase
(EC 3.2.1.2) (Alternate names: 1, 4-α-D-maltohydrolase; glycogenase; sacchoragen amylase)
Another form of amylase, β-amylase is also synthesized by bacteria, fungi and plants. Working from the non reducing end, β-amylase catalyzes the hydrolysis of the second α-1.4 glycosidic bond, cleaving off two glucose units (maltose) at a time.

γ-Amylase
(EC 3.2.1.3) (Alternative names: Glucan 1.4- α-glucosidase; amyloglucosidase; Exo [1.4- α-glucosidase; glucoamylase; lysosomal α-glucosidase, 1.4- α-D-glucan glucohydrolase).
In addition to the last α (1-4) glycosidic linkage at the non reducing end of amylose and amylopectin, yielding glucose, γ-amylase will cleave α (1-6) glycosidic linkage. Unlike the other forms of amylases, γ-amylase is most efficient in acidic environments and has an optimum pH of 3.

2.11.3.3 Enzymatic Hydrolysis of Soluble Starch with an α – Amylase from *Bacillus licheniformis*

The enzymatic hydrolysis of soluble starch with a α-amylase from *Bacillus licheniformis* (commercial enzyme Texamyl 300 L Type DX) have been experimentally studied at pH 7.5, within the temperature range 37-75^{0}C, at an initial substrate concentrations of between 0.25 and 2.00 g/l, and enzyme concentrations of between $0.575*10^{-4}$ and $13.8*10^{-4}$ g/l. We can use the iodometric method for measuring α-amylase activity. The kinetics of the enzymatic hydrolysis was fitted to the Micheallis-Menten equation using the integral method, taking into account that the thermal deactivation of the enzyme follows a second order kinetic. These parameters are fitted to the Arrhenius Equation obtaining activation energies of 24.4 and 41.7 kJ/mol and pre-exponential factors of 734.9g/l and $1.74*10^8$ min^{-1} for K_M and K_1 respectively [14].

These enzymes can be applied to textiles at a pH of 7.5 and within temperature range 37-75^{0}C.

2.11.3.4 The α-amylase family: characteristics and reaction mechanism

Most of the enzymes that convert starch belong to one family based on the amino acid sequence homology: the amylase family or family 13 gylcosyl hydrolases according to the classification of Henrissat. This group comprises those enzymes that have the following features:

(i) They act on α-glycosidic bonds and hydrolyze this bond to produce α-anomeric mono-or oligosaccharides (hydrolysis), form α, 1-4 or 1-6 glycosidic linkages or a combination of both activities.

(ii) They possess a $(\beta/\alpha)_8$ structure containing the catalytic site residues.
(iii) They have four highly conserved regions in their primary sequence which contain the amino acids that form the catalytic site, as well as some amino acids that are essential for the stability of the conserved TIM barrel topology.

The enzymes that match the above criteria and belong to the α-amylase family are listed in

TABLE 6: THE A-AMYLASE FAMILY

Enzyme	EC number	Domains	Main Substrate
Glucan Branching Enzyme	2.4.1.18	A, B, F	Starch glycogen
Amylomaltase	2.4.1.25	A, B1, B2	Starch Glycogen
α -Amylase	3.2.1.1	A, B, C	Starch
Iso-amylase	3.2.1.68	A, B, F, 7	Amylopectin

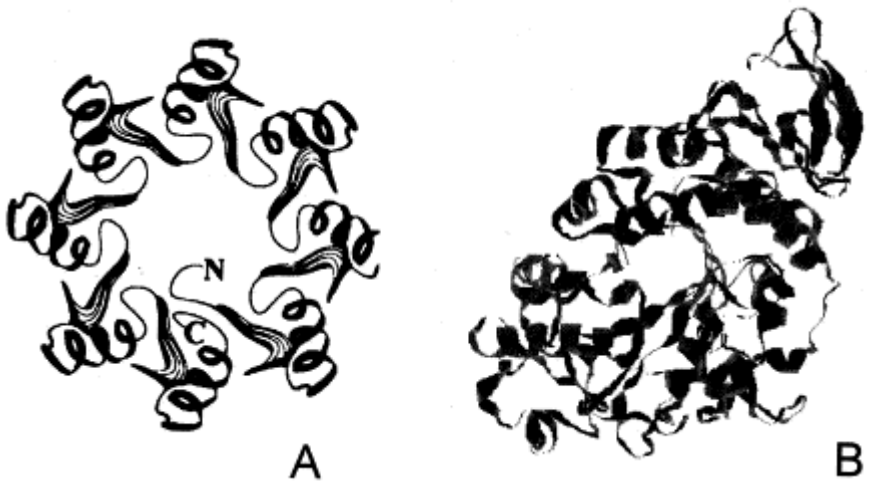


Fig 13: Schematic representation of $(\beta/\alpha)_8$ barrel (A) and 3D structure of α –amylase of *Aspergillus oryzae* or Taka amylase (B), obtained from the Protein Database [16]

2.11.3.5 Utilization of α-amylase family enzymes

- Industrial production of glucose and fructose from starch
- Bakery and anti- staling
- Cyclodextrin/cycloamylose formation

- Miscellaneous applications

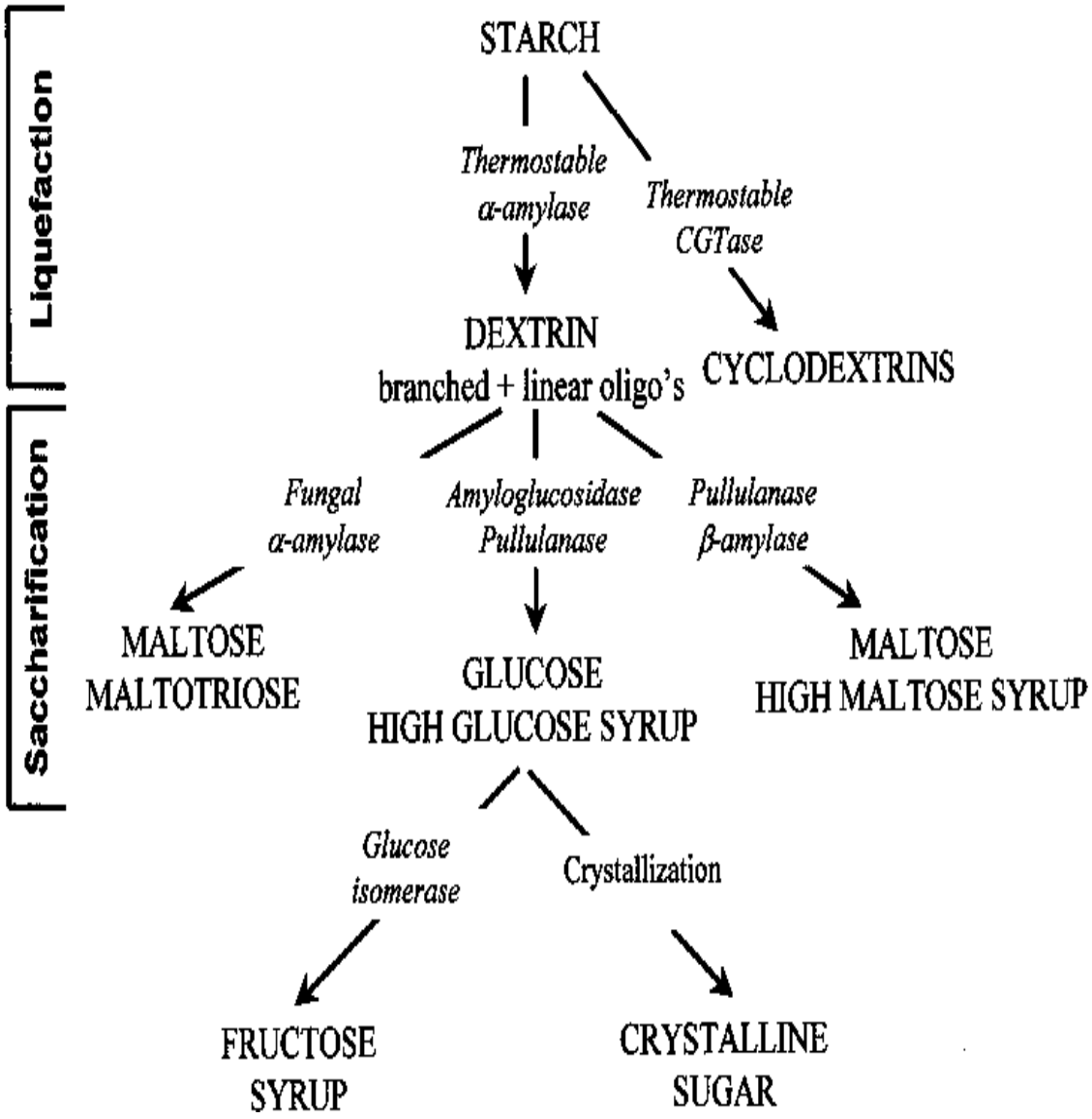


Fig 14: Overview of the industrial processing of starch into cyclodextrins, maltodextrins, glucose or fructose syrups and crystalline sugar.

2.11.3.6 Specifications of amylases are shown on the table below

TABLE 7: SPECIFICATIONS OF AMYLASES

Name	Creamylo 40K	Creamylo HTD	Creamylo MTD	Creamylo LTD	Creamylo BTD
Enzyme	High Temperature Amylase	High Temperature Amylase	Middle Temperature Amylase	Low Temperature Amylase	Amylase
Activity (U/ml)	40,000	20,000 (Actual 2.200)	2000 (Actual 2.200)	2000	4000
Application Temperature (^{0}C)	80-110	80-110	45-70	20-60	20-90
Application pH	5.5-8.0	5.5-8.0	5.5-8.0	5.5-8.0	5.5-8.0

2.11.3.7 Safety

Inhalation of the enzyme mists should be avoided. In case of contact with skin and mucous membranes (eyes, mouth etc), rinse thoroughly with water.
In case of irritation or allergic symptoms, seek medical attention. The details make reference to MSDS.

2.11.4 Pectinase / Pectate lyase

2.11.4.1 Introduction

Pectinases are actually a mixture of enzymes, which along with others such as cellulase, are widely used in the fruit juice industry where they are widely used to help extract, clarify and modify fruit juices. Pectins are large polysaccharide molecules, made up (mainly) chains of several hundred galacturonic acid residues. Enzymes in this pectinase group include polygalacturonases, pectin methyl esterase and pectin lyases. These pectinase enzymes act in different ways on the pectins, which are found in the primary cell walls and in the middle lamella. Pectins are well known also for their ability to form gels.

Pectinases are produced during the natural ripening process of some fruits, where together with cellulases; they help to soften their cell walls. These enzymes are also secreted by plant pathogens such as the fungus *Monilinia fructigena* and the soft-rot bacterium *Erwinia carotovora,* as part of their strategy for penetrating the plant host cell walls. In fact, the products of such enzyme assaults (oligosaccharins) act as a signal which induces uninfected cells to defend themselves [17].

2.11.4.2 Pectinase in textiles

Utilization of highly specific enzymes for various textiles processing applications is gaining momentum because of their ability to replace harsh organic/inorganic chemicals currently used by textile industries. Advancement in biotechnology and enzymology offers the textile industry to reduce the cost of manufacturing of products, protect the environment, address health and safety, and improve quality and functionality. The increasing environmental legislation and burden on the textile industries in the world have forced textile enterprises to seek environmentally friendly processes to replace conventional preparation of cotton fabrics. Wet textile processes consists of desizing, scouring and bleaching in which water is used as the medium to transport of mass and heat across textile materials.

In scouring, the desired hydrophobicity of fabric with high and even wettability can be achieved by removing non-cellulosic material from the fabric especially from the cuticle (waxes and fats) and the primary wall (pectin, protein and organic acids) so that it can be bleached and dyed successfully. Conventional scouring is carried out at higher temperature with caustic soda solutions, which has accompanying disadvantages such as fiber deterioration, high-energy consumption and large amount of strongly basic wastewater pollution.
Nowadays, research has been directed towards the discovery of environment-friendly enzymes that replace these chemical processes of scouring in textile industry [18].

As they are enzymes, pectinases have an optimum temperature and pH at which they are most active. For example, a commercial pectinase might typically be activated at 45 to 55^{0}C and work well at a pH of 4.5 and 5.5.

2.11.4.3 Structure of Pectin

Chemically, pectic substances are complex colloidal acid polysaccharides, with a back bone of galacturonic acid residues linked by α (1-4) linkage. The side chains of pectin molecule consist of L-rhamnose, arabinose, galactose and xylose. The carboxyl groups of galacturonic acid are partially esterifies by methyl groups and partially or completely neutralized by sodium, potassium or ammonium ions. Based on the type of modifications of the backbone chain, pectic substances are classified into protopectin, pectic acid and pectin [18]:

Protopectin: This is a parent pectic substance and upon restricted hydrolysis yields pectin or pectinic acid. Protopectin is occasionally a term used to describe water-insoluble pectic substances found in plant tissues and from which soluble pectic substances are produced.

Pectic acids: These are the galacturonans that contain negligible amounts of methoxyl groups. Normally or acid salts of pectic acid called are pectates.

Pectinic acids: These are the galacturonans with various amounts of methoxyl groups. Pectinates are normal or acid salts of pectinic acids. Pectinic acids alone have the unique property of forming a gel with sugar and acid or, if suitably low in methyl content, with certain other compounds such as calcium salts.

Pectins: A generic name for the mixture of widely differing compositions containing pectinic acid as the major component. Pectin in native form is located in the cell wall and it may be interlined with other structural polysaccharides and proteins to form insoluble protopectin.

2.11.4.4 Classification of pectic enzymes

Pectinases are classified under three heading according to the following criteria:

- whether pectin, pectin acids the preferred substrate,
- whether pectinases act by trans-elimination or hydrolysis and
- whether the cleavage is random or endwise.

The three major types of pectinases are as follows:

2.11.4.4.1 Pectinesterases

They are also known as pectinmethyl hydrolase, catalyzes de-esterification of the methoxyl group of pectin forming pectic acid. The enzyme acts preferentially on a methyl ester group of galacturonate unit next to a non-esterifies galacturonate unit.

2.11.4.4.2 Depolymerizing enzymes

These are the enzyme:

(i) Hydrolyzing gylcosidic linkages

They include:

(a) Polymethylgalacturonases (PMG), catalyze the hydrolytic cleavage of α-1-4 glycosidic bonds.

(b) Polygalacturonases (PG), catalyze hydrolysis of α-1-4 glycosidic linkages in pectic acid (polygalacturonic acid).

(ii) Cleaving

Cleaving α-1-4 glycosidic linkages by trans –elimination, which results in galacturonide with unsaturated bond between C4 and C5 at the non-reducing end of the galacturonic acid formed. These include:

(a)Polymethylegalacturonate lyases (PMGL), catalyze breakdown of pectin by trans-eliminate cleavage.

(b) Polygalacturonate lyases (PGL), catalyze cleavage of α-1-4 glycosidic linkages in pectic acid by trans-elimination.

2.11.4.4.3 Protopectinase

This enzyme solubilizes propectin forming highly polymerized soluble pectin. On the basis of their applications, pectinases are mainly of two types: ***Acidic pectinases*** and ***Alkaline pectinases*** [19].

2.11.4.5 Characterization of microbial pectinases are shown on the table below

TABLE 8: CHARACTERIZATION OF MICROBIAL PECTINASES

Producer	Type of Pectinase	Optimum pH for Activity	Optimum Temperature for Activity (50^0C)	Reference
Acidic Pectinase				
Aspergillus niger CH4	Endo-pectinase, pectinase	4.5-6.0	Below 50	Acuna-Arguelles et al., 1995
Penicillium frequentans	Endopolygalacturonase (Endo-PG)	4.5-4.7	50	Borin et al.,1996
Sclerotium rolifsii	Endo-PG	3.5	55	Channe and Shewal, 1995
Rhizoctonia solani	Endo-PG	4.8	50	Marcus et al., 1987
Clotridium thermosaccharolyticum	Polygalacturonate Hydrolase	5.5-7.0	30-40	Rijssel et al., 1993
Alkaline Pectinase				
Penicilium italicum CECT 22941	Pectin lyase	8.0	50	Alan et al., 1990
Bacillus sp. DT 7	Pectin lyase	8.0	60	Kashyap et al., 2000
Pseudomonas syringae pv. Glycinea	PAL	8.0	30-40	Magro et al., 1994
Bacillus sp. NT-33	PG	10.5	75	Cao et al., 1992

2.11.4.6 Application of Pectinases in Textile Industry

Pectinases have been used in conjunction with amylases, lipases, cellulases and hemicellulases to remove sizing agents from cotton in a safe and eco-friendly manner, replacing toxic caustic soda used for the purpose earlier. Bioscouring is a novel process for removal of non-cellulosic impurities from the fiber with specific enzymes. Pectinases have been used for this purpose without any negative side effect of cellulose degradation.

In this, pectinase destroy the cotton cuticle structure by digesting the pectin and removing the connection between the cuticle and the body of cotton fibers whereas cellulase can destroy

cuticle structure by digesting the primary wall cellulose immediately under the cuticle of cotton [20].

- ✓ Biological Oxygen Demand (BOD) of enzymatic scouring process is 20-45% as compared to alkaline scouring (100%).
- ✓ Chemical Oxygen Demand (COD) of enzymatic scouring process is 20-45% as compared to alkaline scouring (100%).
- ✓ Total Dissolved Solids (TDS) of enzymatic scouring process is 10-50% as compared to alkaline scouring (100%).
- ✓ Handle is very soft in enzymatic scouring compared to harsh feel in alkaline scouring process [20].

2.11.4.7 Specifications of pectinases are shown on the table below

TABLE 9: SPECIFICATIONS OF PECTINASES

Name	Crepectinase	Crepectinase Lipase Conc.	Crepectinase Alkaline	Crepectinase Acid
Enzyme	Lipase Pectinase	Lipase Pectinase	Alkaline Pectinase	Alkaline Pectinase
Activity (IU/ml)	300	3000	300	1000-5000
Customizable Activity	10,000-100,000		300-2000	
Application Temperature (^{0}C)	45-55	45-55	45-50	30-50
Application pH	8.0-9.0	8.0 - 9.0	6.0-10.0	4.5-6.0

2.11.4.8 Safety

In case of contact with skin and mucous membrane (eyes, mouth, etc.), rinse thoroughly with water.
In case of irritation or allergic symptoms, seek medical attention. The details make reference to MSDS.

2.11.4.9 Storage

Being stored under cool condition is recommended.

2.11.5 Laccase

2.11.5.1 Introduction

Laccases are copper-containing oxidase enzymes that are found in many plants, fungi and microorganisms. The copper is bound in several types; Type 1, Type 2 and Type 3. The ensemble of types 2 and 3 copper is called a trinuclear cluster (see fig. 15). Laccases act on phenols and similar molecules, performing one-electron oxidations, which remain poorly defined. It is proposed that laccases play a role in the formation of lignin by promoting the oxidative coupling of lignols, a family of naturally occurring phenols [22].

Laccases can be polymetric, and the enzymatically active form can be a dimer or trimer. Other laccases such as one produces by the fungus Pleurotus Ostreatus, play a role in the degradation of lignin and can therefore be included in the broad category of ligninases. Spectrophotometry can be used to detect laccases, using the substrates ABTS, syringaldazine, 2.6 – dimethoxyphenol and dimethyl-p-phenylenediamine. Activity can also be monitored with an oxygen sensor, as the oxidation of the substrate is paired with the reduction of oxygen to water.

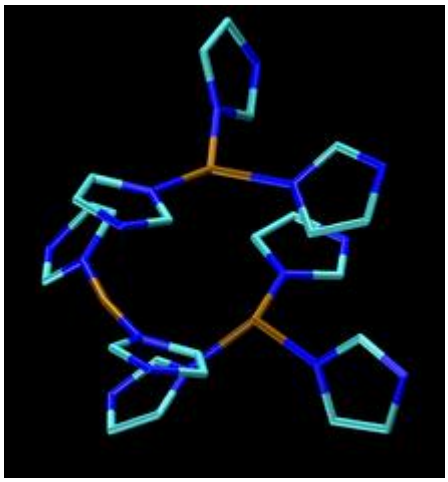


Fig. 15: The tricopper site found in many laccases, notice that each copper center is bound to imidazole (color code: copper is brown, nitrogen is blue)

Laccases are some of the few oxidoreductases commercialized as industrial catalysts. The enzymes can be used for ***textile dyeing / textile finishing,*** wine cork making, teeth whitening and many other industrial, environmental, diagonistic and synthetic uses. Laccases can be used in Bioremediation. Protein ligand docking can be used to predict the putative pollutants that can be degraded by laccase [22].

2.11.5.2 Application of Laccases in the Textile Industry

Laccases in combination with redox mediators are used in textile processing to bleach denim fabrics, decolorizing indigo. Research efforts have been made to use laccase as a bleaching and or oxidative coupling agent for dyeing animal fibers and human hair. Laccases are unspecific oxidoreductases which catalyze the removal of hydrogen atom from the hydroxyl group of *ortho* and *para*-substituted mono and polyphenolic substrates and from aromatic amines by one-electron abstraction while the co-substrate oxygen is reduced yielding water. Free radicals formed in this reaction from the substrates that are capable of undergoing further depolymerisation, repolymerisation, demethylation or quinone formation [5].

2.11.5.3 Structure and Classification

Laccases E.C 1.10.3.2, p-diphenol: dioxygen oxidoreductase are a group of multi-copper containing enzymes that catalyze one-electron oxidation of phenolic compounds with concomitant reduction of oxygen to water, and whose active sites is similar to that of ascorbatoxidase, ceruloplasmin and bilirubin oxidase. All fungal laccases are glycoproteins (Mayer & Staples, 2002). The catalytic site of laccase is quite conserved among different species, but the rest of the enzyme structure shows high diversity.

Fungal laccases are mostly inducible, extracellular, monomeric glycoproteins with carbohydrate contents of 8%-50% (Heinzkill et al., 1996) and are multinuclear enzymes (Gayazov and Rodakiewicz-Nowak, 1996). Typically molecular mass of fungal laccases is from 60 to 80 kDa (Heinzkill et al., 1998) [23].

Laccases from the same fungal species were reported to contain 49-41% reducing sugar content (Slomczynski et al., 1995). The tertiary structure of laccase has been determined by X-rays crystallography. Typically the active site of laccase comprises four Cu atoms in three groups. Cu atoms differ from each other in their electron paramagnetic resonance (EPR) signals. Type 1 Cu exists in its oxidized form and is responsible for the blue colour of the enzyme at 600nm and is EPR detectable. Type 2 Cu is colorless, but EPR detectible. Type 3 Cu exhibits a weak absorption at 600nm but has no EPR signal. The type 2&3 Cu site is closely together and forms a trinuclear centre that is involved in the catalytic mechanism of the enzyme action. Almost all fungi that have been examined produce more than one isoform of laccase.

Redox potential (RP) of fungal laccases range from 0.4 V to 0.8 V. Fungal laccases with RP between 0.4 and 0.6 V belong to the “Low RP” group such as Coprinus cinereus whereas those with a RP between 0.6 and 0.8 V are referred as “High RP” laccase such as Trametes versicolor.

2.11.5.4 Mode of Action and Mediators

Laccase only attacks the phenolic compounds leading to Cα oxidation, Cα-Cβ cleavage and aryl-alkyl cleavage. Laccases are able to reduce one molecule of di-oxygen to two molecules of water while performing one electron oxidation of a wide range of aromatic compounds which includes polyphenols (Bourbonnais and Paice, 1996), methoxy-substituted *Fungal Laccases* 77 monophenols and aromatic amines (Bourbonnais et al., 1995). This oxidation results in an oxygen-centered free radical, which can then be converted in a second enzyme-catalyzed reaction to quinine. Laccase catalysis is agreed to take place in three steps:

1. Type I Cu reduction by substrate.
2. Electron transfer from type I Cu to the type II Cu and type III Cu trinuclear cluster.
3. Reduction of oxygen to water at the trinuclear cluster (Gianfreda et al., 1999).

The substrate range of laccase can be extended to non-phenolic subunits by the inclusion of mediators. Mediators are a group of low molecular-weight organic compounds that can be oxidized by laccase first and from highly active cation radicals to reach with carious chemicals including non-phenolic compounds that laccase alone cannot oxidase (Figure 16). The most common synthetic mediators are 1-hydroxybenzotriazole (HOBT), N-hydroxyphthalimide (NHPI) and 2, 2'-azinobis-3-ethylthiazoline-6-sulfonat (ABTS) (Figure 17) [23].

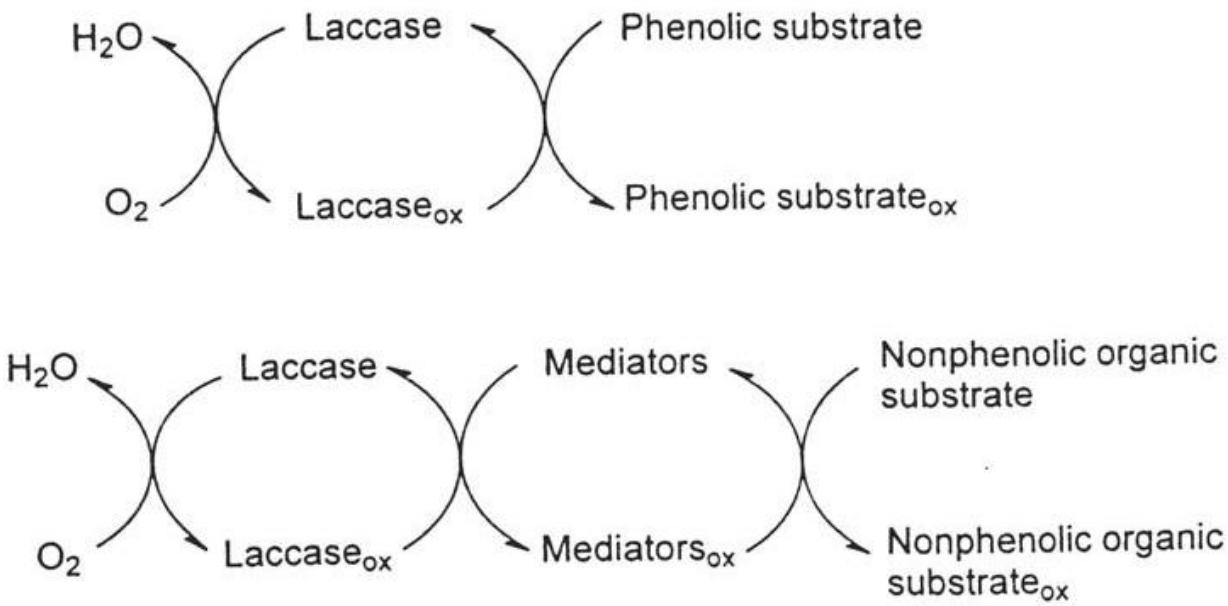


Fig. 16: A comparison of catalytic mechanism of laccase between with and without mediators

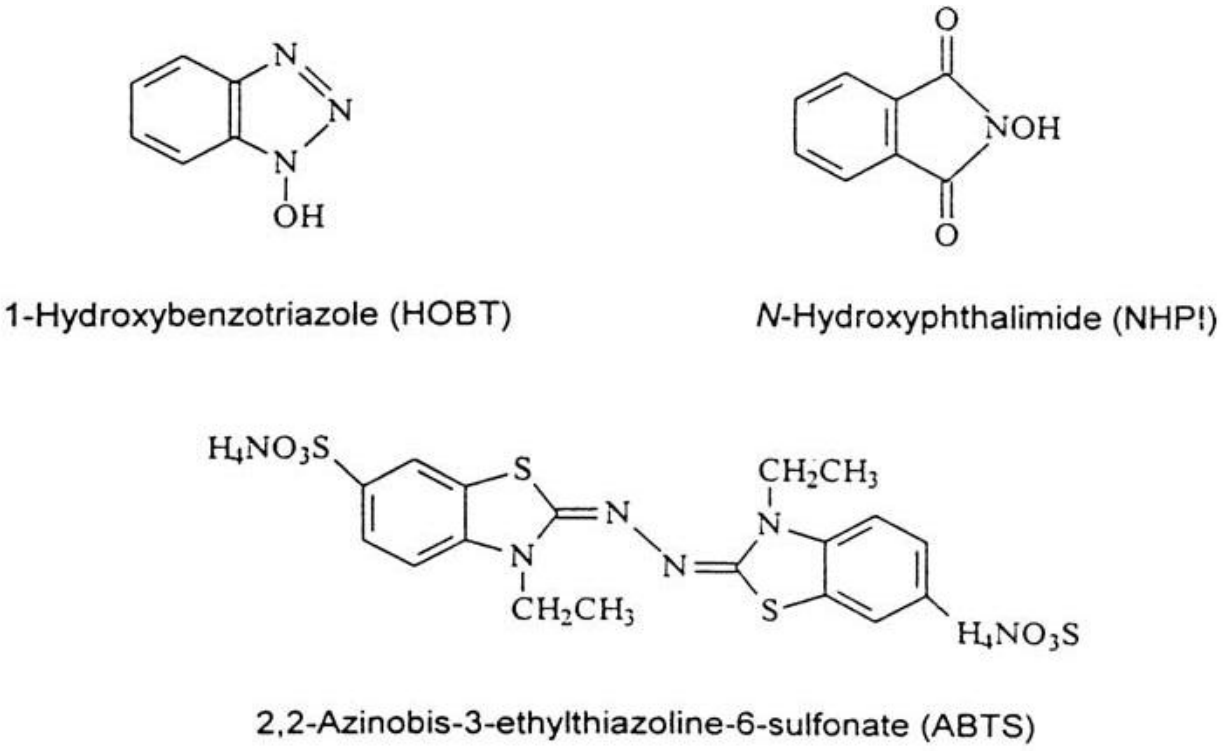


Fig. 17: Structures of three common synthetic mediators

2.11.5.5 Influence of pH and temperature on laccase production

There is not much information published on the influence of pH on laccase production, but most reports indicated initial pH levels set between pH 4.5 and pH 6.0 prior to inoculation (Thurston, 1994). The levels are not controlled during most cultivation. It has been found that the optimal temperature for laccase production is between 25°C and 30°C *(Pointing et al., 2000)*. When cultivated fungi at temperatures higher than 30°C the activity of lignolytic enzymes was reduced *(Zadrazil et al., 1999).*

2.11.6 Catalase

2.11.6.1 Introduction

Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, when it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second.

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with hydrogen peroxide. The optimum pH for human catalase is approximately 7.0 and has a fairly broad maximum (the rate of reaction does not change appreciably at pH's 6.8 and 7.5). The pH optimum for other catalases varies between 4 and 11 depending on the species. The optimum temperature also varies by species [5][6][21].

2.11.6.2 History

Catalase was first noticed as a substance in 1818 when Louis Jacques Thenard, who discovered H_2O_2 (hydrogen peroxide), suggested that its breakdown is caused by a substance. In 1900, Oscar Loew was the first to give it the name catalase, and found its presence in many plants and animals. In 1937, catalase from beef liver was crystallized by James B. Sumner and the molecular weight was worked out in 1938.
In 1969, the amino acid sequence of bovine catalase was worfed out. Then in 1981, the 3D structure of the protein was revealed.

2.11.6.3 Description

Crecatalase is a concentrated catalase (E.C 1.11.1.6) that catalyses the decomposition of hydrogen peroxide into water and molecular oxygen. It can be used to remove residual hydrogen peroxide in applications where hydrogen peroxide is added e.g. pasteurization or bleaching.
Thus, it has suggested using catalase in the textile industry for the removal of hydrogen peroxide from fabric which is bleached by an alkaline hydrogen peroxide treatment before dyeing.

2.11.6.4 Decomposition of H_2O_2

Catalase disproportionates:

$$2H_2O_2 \text{ to } 2H_2O + O_2$$

H_2O_2 is a powerful oxidizing agent and is potentially damaging to cells. By preventing excessive H_2O_2. build up. Catalase allows important cellular processes which produce H_2O_2 as a by-product to take place safely.

2.11.6.4.1 The Peroxidative Reaction

Catalase performs a very elegant 'reshuffling' of toxic compounds. In the following peroxidative reaction, a second family of reactions catalyzed by catalase, possibilities for the compound RH2 includes phenols, formic acid, formaldehyde and alcohols:

$$H_2O_2 + RH_2 \text{ to } 2H_2O + R,$$

The trick of taking toxins and the potentially harmful H_2O_2 and recombining them to produce harmless or useful products and water seemed to be a very neat one. Unfortunately details are

scarce. I have not yet found details of Catalyses' specificity for different RH_2, or how readily it catalyses peroxidative reactions as compared to catalyzing hydrogen peroxide disproportionation [5][6][23][21].

2.11.6.4.2 Molecular mechanism

While the complete mechanism of catalase is not currently known, the reaction is believed to occur in two stages:

$$H_2O_2 + Fe(lll)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E(.+) \quad (6)$$

$$H_2O_2 + O{=}Fe(IV)\text{-}E(.+) \rightarrow H_2O + Fe(III)\text{-}E + O_2 \quad (7)$$

Here Fe()-E represents the iron center of the heme group attached to the enzyme. Fe(IV)-E(.+) is a mesomeric form of Fe(V)-E, meaning that iron I not completely oxidized to +V but receives some "supporting electrons" from the heme ligand. This heme has to be drawn then as radical cation (.+).

As hydrogen peroxide enters the active site, it interacts with the amino acids, causing a proton (hydrogen ion) to transfer between the oxygen atoms. The free oxygen atom coordinates, freeing the newly-formed water molecule and Fe(IV)=O. Fe(IV)=O reacts with a second hydrogen peroxide molecule to reform Fe(III)-E and produce water and oxygen. The reactivity of the iron center may be improved by the presence of the phenolate ligand in the fifth iron ligand (a ligand is an ion or molecule (see also: functional group) that binds to a central metal atom to form a coordination complex), which can assist in the oxidation of the Fe(III) to Fe(IV). The efficiency of the reaction may also be improved by the interactions with reaction intermediates. In general, the rate of the reaction can be determined by the Michaeli-menten equation.

Catalase can also oxidize different toxins, such as formaldehyde, formic acid, phenols and alcohol as mentioned above (peroxidative reaction).

Any heavy metal ion (such as copper cations in copper(II)sulfate) will act as a non-competitive inhibitor on catalase. Also, the poison cyanide is a competitive inhibitor of catalase, strongly binding to the heme of catalase and stopping the enzyme's action [5][6][21].

2.11.6.5 Specifications of catalases are shown on the table below

TABLE 10: SPECIFICATIONS

Name	Crecatalase	Crecatalase Conc.
Enzyme	Catalase	Catalase
Activity	50,000 (Amano)	200,000 (Amano)
Customizable Activity	50,000-200,000	
Application Temperature (^{0}C)	30-60	30-60
Application pH	6.5-8.5	6.5-7.5
Commercial Equivalent	Teminox Ultra 50L (Novo) Oxygone T 100 (Genencor)	Teminox Ultra 200L (Novo) Oxygone T100 (Genencor)
Remarks	Can dilute 3-20 times	Can dilute 10 times

2.11.6.6 Safety

Inhalation of the enzymes mists should be avoided. In case of contact with skin and mucous membranes such as eyes, mouth etc, rinse thoroughly with water. In case of irritation or allergic symptoms, seek medical attention.
The details make reference to MSDS [21].

2.12 Enzyme Applications in Textile Wet Processing

Some of the important commercial applications of enzymes are reviewed here with special emphasis on their environmental performance.

2.12.1 Biopolishing

Cotton and other natural fabrics based on cellulose can be improved by an enzymatic treatment known as biopolishing. As the name suggests, the treatment gives the fabric a smoother and glossier appearance.
Cellulases hydrolyze the microfibrils (hairs or fuzz) protruding from the surface of the yarn. A ball of fuzz is called a 'pill' and these pills can present a serious quality problem since they result in an unattractive knotty fabric appearance. After biopolishing, fabric shows a much lower pilling tendency. The other benefits of removing fuzz are a softer and smoother handle, and better colour brightness. An alternative way to carry out polishing is by singeing with a gas flame and therefore the use of enzymes saves gas and emissions from the combustion process.

Cellulase enzymes are a class of hydrolytic enzymes that are used for different cotton finishing processes: cellulases can be utilized to give indigo-dyed cotton fabrics (denim) an aged appearance (also known as biostoning), and to give cotton fabrics a renewed appearance by colour brightening and softening of the material through the removal of microfibers (depilling, also known as biopolishing). Cellulase is a typical multi-component enzyme that consists of mainly:

- Endoglucanases (EGs), (EC 3.2.1.4);
- Cellobiohydrolases (CBHs) or exo-cellobiohydrolases, (EC 3.2.1.91);
- b - glucosidases or cellobiases (EC 3.2.1.21) [24] [5].

2.12.2 Bioscouring of cotton

Before grey cotton can be dyed and finished, it has to be treated in order to make it hydrophilic and to remove the primary cell wall. In conventional cotton scouring processes, high temperatures (90^0-100^0C) and high concentrations of NaOH (approx. 1mol/L) are used to remove the primary cell wall (pectin, protein, organic acids) and hydrophobic components from the cuticle (waxes and fats) in a non specific way to make the fibre hydrophilic.
Owing to the high concentration of NaOH, extensive washing and rinsing is required, causing increase water consumption. The use of high concentrations of NaOH also requires the neutralization of the wastewater, which requires additional chemicals.
It is obvious that this process needs to be improved considerably to meet today's energy and environmental demands. Much research has been directed to replace this process with the enzymatic one. The potential to degrade and remove the undesired components from the cotton fibres of different enzymes, such as ***pectinases, cellulases and lipases,*** as well as different process conditions, have been investigated.

Novozymes, Bayer and Dexter Chemical Corporation have introduced an enzymatic alternative for scouring woven and knitted cotton fabrics in the textile industry on the basis of an alkaline pectinase (EC4.2.2.2) produced by a genetically modified *Bacillus* strain. On an industrial scale, the bioscouring process using alkaline pectinases has been performed successfully in pad-batch and continuous processes, and integrated desizing in batch and continuous processes [5].

Process

The idea is that pectin acts as a sort of cement or matrix that stabilizes the primary cell wall of the cotton fibres. During incubation the enzymes will degrade pectin, thereby destabilizing the structure in the outer layers. The weakened outer layers can be removed in a subsequent wash process. The bioscouring process results being softer than those scoured in the conventional NaOH process, however, the degree of whiteness is often less and the process is not suitable for removing seed coat fragments and motes adequately.

A typical temperature for the enzymatic scouring of woven and knitted cotton fabrics in a jet machines is between 70^0- 80^0C. The liquor ratio is 8:1 and the enzyme dosage should be between 0.5 and 1.0%. These conditions are applicable to most exhaustion machinery. Wetting agents and nonionic surfactants should be added together with the enzyme to enhance enzyme penetration and adsorption, fibre swelling and the removal of waxes. A buffer is needed e.g. a phosphate or citrate buffer, to maintain the pH between 7 and 9.5 (optimal pH 8.5-9.0). Combined with desizing, the pH should not exceed 7.5-8.0.
The Ca2+ concentration is an important parameter in the enzymatic process. Its presence slows down the degradation of pectin but stabilizing the enzyme. Therefore the addition of strong chelators is recommended only for the extraction and washing/rinsing phase.

2.12.3 Desizing of cotton

During weaving, warp yarns are exposed to considerable mechanical strains. To prevent the yarns from breaking, they are coated with a sizing agent (a protective glue and lubricant).The sizing agent is most often based on starch.
Apart from starch, synthetic sizing agents are available, for example polyvinyl alcohol (PVALc), but, for economic reasons, starch is still the most favorable sizing agent. After weaving the fabric, the sizing agent needs to be removed since it hinders textile-finishing processes such as dyeing. This desizing process used to be done chemically using, for example, hydrogen peroxide, (H_2O_2) and sodium hydroxide (NaOH), but since the 1950s enzymatic desizing processes based on a-amylases have been widely introduced and implemented successfully in the textile industry. In the enzymatic desizing process an almost complete removal of starch-containing size is obtained without any fibre damage. Amylases were derived from mulds or pancreas but are nowadays produced by bacteria (especially *Bacillus subtilis*).

Process

Biotechnological progress and genetic engineering for micro-organisms have allowed thermostable enzymes to be widely available nowadays, and therefore different temperatures, ranging from 20^0 to 80^0C for conventional α-amylases and 40 to 110°C for thermostable α-amylases, are applicable. The optimum pH lies between 4 and 10 depending on the enzyme used. Most α-amylases are suitable for all common batch and (semi-)continuous processes, such as jet, jig, cold and hot pad-batch (see figure 18), pad-steam and J-box

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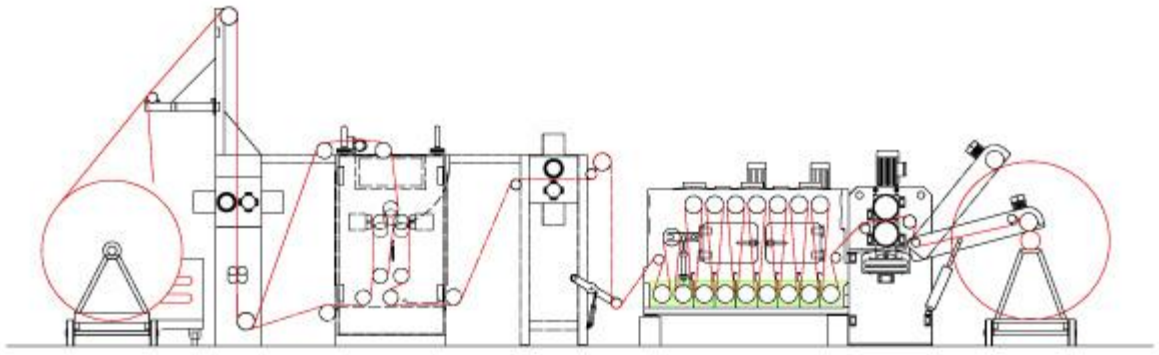


Fig. 18: Pad batch processes typically used in the enzymatic desizing of cotton fabrics.

During impregnation, the hot water causes the starch to gelatinize and the fabric becomes fully wetted and impregnated with the enzyme solution. The average liquid take-up during impregnation is approximately 1L/kg fabric, and depends on the characteristics of the fabric such as porosity and the additives present in the impregnation liquor. Chelating agents should preferably not be used during desizing process because calcium ions (at ppm level) stabilize the enzymes. Wetting agents and non-ionic surfactants can be used to enhance enzyme penetration and adsorption, fibre swelling and to promote the removal of waxes, soils and synthetic sizing agents. Non-ionic surfactants are suitable for combination with enzymes, whereas anionic and cationic surfactant may inactivate the enzyme through denaturation. Lubricants are generally recommended to be used in combination with a-amylases during desizing, especially in jets and rotary washers, to reduce the formation of crease marks and streaks. After the enzymatic treatment, fabrics should be washed off above 80°C, often between 90 to 100°C, in alkaline liquor followed by a wash in neutral liquor [24] [5].

2.12.4 Denim Abrasion and Finishing

Many denim garments are subjected to a wash treatment to give them a worn look. In the traditional stonewashing process, the blue denim is faded by the abrasive action of pumice stones. Cellulases were first introduced in the 1980s and nowadays more than 80% of denim finishers use cellulases or a combination of stones and cellulases to create the worn look on denim. Cellulases work by loosening the indigo dye on the denim in a process known as 'biostoning'. A small dose of enzyme replaces several kilograms of pumice stones so the handling of enzymes is easier. The use of fewer stones results in less damage to the garments, less wear on machines and less pumice dust in the laundry environment. Approximately 1 kg of stones is required to stonewash 1 kg of jeans. In a 1-hour wash cycle, the pumice stones will lose up to 50% of their weight. The pumice grit can block drains so it needs to be filtered out from the wastewater. Large amounts of pumice sludge can be produced. For example, a denim finisher

processing 100 000 garments a week with stones typically generates 18 tons of sludge (BioTimes, 1997). An environmental assessment was performed on jeans (OECD, 1998). The conclusions were that the biostoning process proved to be more environmentally sound than the traditional stoning process using pumice [24].

2.12.5 Bleach clean-up

Natural fabrics such as cotton are normally bleached with hydrogen peroxide before dyeing. Bleaches are highly reactive chemicals and any peroxide left on the fabric can interfere with the dyeing process. Therefore a thorough 'bleach clean-up' is necessary. Enzymatic bleach clean-up was first launched in the 1980s. The traditional method is to neutralize the bleach with a reducing agent or to rinse with hot water. Both the methods require copious amounts of water. Enzymes represent a convenient alternative because they are easier and quicker to use. A small dose of catalase is capable of decomposing hydrogen peroxide to water and oxygen. Compared with the traditional methods of removing bleach, the enzymatic process results in less polluted wastewater and/or reduced energy and water consumption, depending on the process that enzymes replace. Most of the chemical agents used for the neutralization of residual bleach are hazardous to handle and problematic to the environment. These reducing
agents also need to be adequately removed prior to dyeing. Residual reducing agents can have a detrimental effect on shade reproducibility from batch to batch. In contrast, enzymes can be handled safely, have no effect on dye and can even be used in the same bath as dyeing. Furthermore, they are completely degradable in nature [5] [24].

2.13 Future Trends and Applications

Another aim of this study is the combining of different enzymatic processes in the same bath which will help to save more water and streamline processing to save time and ultimately costs.

2.13.1 Combined bleach clean-up and dyeing

Already the use of a catalase enzyme for bleach clean-up allows mills to reduce at least one process step by adding the catalase to the dyebath, thereby allowing the removal of hydrogen peroxide to take place in the same bath as dyeing. The hydrogen peroxide is first removed in a quick 5–10 minute step followed by dyeing in the same bath. This is made possible by the fact that the catalase only targets the hydrogen peroxide as a substrate to neutralize. It does not target the dyes and these are therefore unaffected.

2.13.2 Combined dyeing and bio-polishing

In April 2008, Novozymes launched a new formulated product called Cellusoft Combi that allows mills to carry out combined biopolishing and dyeing in the same bath. Traditionally, biopolishing is an additional process and this adds extra processing costs to a standard dyeing process. In traditional cases, the biopolishing process will be carried out before or after dyeing. This typically adds 90–120 minutes to the complete process. With Cellusoft Combi, this entire process is eliminated by carrying out the biopolishing process in the dyebath itself. Simply adjust the pH for dyeing after bleaching, add the Cellusoft Combi and continue dyeing. Carrying out biopolishing with Cellusoft Combi in the same bath as dyeing means savings in water, energy and process time.

Benefits

- **Reduce processing time by up to 50%**
  Increasing production throughout

- **Fewer drops and fills results in:**
  Reduced water consumption (3000 litres for 100Kg Cotton)
  Reduced effluent volumes (3000 litres for 100Kg Cotton)
  Reduced energy consumption

- **Broad pH means**
  Robust processing conditions
  Excellent batch to batch reproducibility
  Ease of use (enzyme deactivation by default in dyeing – alkali dosing)

- **Using Cellusoft Combi means**
  One product carrying out two operations in one

- **Significant removal of dead cotton**

TABLE 11: PROCESSING CONDITIONS OF CELLUSOFT COMBI

Fabric Type	Cotton and cotton blends
Equipment	Jets and other high mechanical action equipment
Liquor Ratio	1:4 – 1:20
pH	5 – 8 (6 – 7 for combined dyeing and biopolishing)
Temperature	40 - 60^0C (best @ 50 – 60^0C)
Dosage	1 – 3 %
Processing time	45 – 90 minutes
Inactivation	During alkali dosing in reactive dyeing

2.13.3 Combined biopolishing and bioscouring

A similar concept is to combine the bioscouring and biopolishing processes in the same bath prior to dyeing. Here Scourzyme L and Cellusoft CR from Novozymes are added together at the start of the process. This is made possible by the fact that both these products have overlapping temperature and pH profiles. Once again, this saves process time, water, effluents and energy. There is also a synergistic effect when combining these two products resulting in even better biopolishing and bioscouring effects than if these products were used separately.

2.13.4 Combined desizing and bioscouring

Novozymes has launched the concept of CDB (combined desizing and bioscouring). This allows the enzymatic scouring and desizing of fabric to be performed in one step. Traditionally, desizing is carried out as a separate step where an amylase is applied to the fabric by means of pad batch or exhaust. In most cases woven fabrics that need to be desized are treated by means of one of the so-called pad batch methods where the fabric is allowed to dwell for several hours. Alternatively this is done on a continuous basis at high temperatures. With Scourzyme L and Aquazym SD-L, it is now possible to carry out desizing and bioscouring in one step. The pH and temperature profiles for these two products overlap and this allows for combining the processes of desizing and bioscouring in pad batch conditions. The combination of these enzymes allows mills to omit the conventional strong alkaline scouring process. Instead of having a traditional three-step process (desize–scour–bleach), it is possible to reduce this to a two-step process (CDB–bleach). This saves large amounts of energy, water and effluent
while increasing production capacity [5] [6] [10] [24].

2.14 Longer- term Perspectives

There is a huge potential to reduce energy costs in the pre-treatment processes in a textile mill. For example, the process of bleaching needs to be addressed in future in order to reduce energy costs and enhance the quality of bleached fabric. Enzymes could provide the answer. It should be mentioned that the vast majority of enzyme applications today are used for the
treatment of cotton. The textile industry is also looking for new ways to deal with current problems on fibres other than cotton. This includes finding ways to improve the quality of the so-called bast fibres (hemp, linen, etc.), wool and even synthetic fibres. The search is on for industrial enzymes that are commercially viable for use on these substrates. There is also a need to find alternative ways of dealing with an array of different sizing agents other than starch. Enzymes could even be used in future to break down dyestuffs in the effluents from dyehouses and denim finishing laundries. Dyestuffs remain one of the most difficult substances to remove at an effluent treatment plant. With all these opportunities and the need to move towards sustainable development, industrial enzymes could provide some of the solutions the textile industry is looking for in the future.

3. Experimental Part

3.1 Enzymatic Desizing of Cotton Fabrics

Enzymatic desizing is the classical desizing process of degrading starch size on cotton fabrics using enzymes. Enzymes are complex organic, soluble bio-catalysts, formed by living organisms that catalyze chemical reaction in biological processes. Enzymes are quite specific in their action on a particular substance. A small quantity of enzyme is able to decompose a large quantity of the substance it acts upon. Enzymes are usually named by the kind of substance degraded in the reaction it catalyzes. Amylases are the enzymes that hydrolyze and reduce the molecular weight of amylose and amylopectin molecules in starch, rendering it water soluble enough to be washed off the fabric. Effective enzymatic desizing requires strict control of pH, temperature, water hardness, electrolyte addition and choice of surfactant.

Testing No 1 (100% Woven Cotton)

3.1.1 Aim

1. To desize 100% cotton (knitted and woven) that was sized by natural starch and to calculate the amount of starch left in the cotton after desizing so as to figure out how much starch is left in the fabric or is totally desized. After desizing, it will be easy to know that certain fabric is suitable for dyeing or not.
2. Proof of starch before desizing, also determine the degree of degradation of starch with the help of iodine solution.
3. Desizing of samples
4. Proof of starch after desizing, again to determine the degree of degradation of starch with the help of iodine solution.

3.1.2 Material

100% cotton fabric	(woven)
Texamyl	(enzyme)
Sodium chloride	(Salt)
Slovafol 909	(nonionic wetting agent)
Temperature	80-90^{0}c
Liquor Ratio	1:100
Sample size	10*20cm
Weight of the sample 1.7g	
Time	30 minutes

3.1.3 Method

Prepare 4 samples for different pH's **3, 5, 8 & 10**
Prepare 6 samples for different amount of enzyme and compare results **0, 5; 1; 2; 3; 5 & 8 (g/l)**
(All pH 6.5)

3.1.3.1 For different pH's (1)

Total bath - 100*1.7=**170ml**

3g/l Texamyl	1g/l Slovafol 909	1g/l NaCl	Stock Solution
3g…….1000ml	1g…….1000ml	1g…….1000ml	50g…….1000ml
Xg…….170ml	xg…….170ml	xg…….170ml	0.17g….xml
X =**0.51g**	x =**0.17g**	x =0.17g	x =**3.4ml**

Both Texamyl and Slovafol are concentrated.

For 4 samples we have:
Texamyl =**2.04g**
Slovafol =**0.68g**
NaCl =**13.6ml**
L.R =**170*4**
Total bath =**680ml (4 samples)**

Prepare 4 beakers and put all the chemicals and start to heat up to 85^{0}C for 30 minutes.

3.1.3.2 For different amounts of Texamyl (2)

0.5g……..1000ml
Xg……. 170ml
X = **0.085g**

1 = **0.17g**
2 = **0.34g**
3 = **0.51g**
5 = **0.85g**
8 = **1.36g**

At pH 6.5

The Slovafol, NaCl and LR have same amount as **1.**
Prepare 6 beakers and put all the chemicals and start to heat up to 85^{0}c for 30 minutes.

We adjust the pH by using Ammonium Hydroxide **NH_4OH.**
We test the starch by the use of **Iodine (I_2).**
After 30 mins offload the pots, dry the samples and check the results.

3.1.4 Results

TABLE 12: SAMPLES WITH DIFFERENT PH FOR 30 MINUTES

Sample	pH	Color	Starch
1	3	Violet-purple	Partly degraded
2	5	Yellow	No starch
3	8	Yellow & little green	No starch
4	10	Yellow & little green	No starch

TABLE 13: SAMPLES WITH DIFFERENT AMOUNT OF TEXAMYL FOR 30 MINUTES

Sample	Texamyl (g/l)	Color	Starch
1	0.5	Yellow	No starch
2	1	Yellow	No starch
3	2	Yellow	No starch
4	3	Yellow	No starch
5	5	Yellow	No starch
6	8	Yellow	No starch

NB: The procedure was the same as for 30 minutes and the results were as follows:

TABLE14: SAMPLES WITH DIFFERENT PH FOR 20 MINUTES

Sample	pH	Color	Starch
1	3	Violet-purple	Partly degraded
2	5	Yellow	No starch
3	8	Yellow & little green	No starch
4	10	Yellow & little green	No starch

TABLE 15: SAMPLES WITH DIFFERENT AMOUNT OF TEXAMYL FOR 20 MINUTES

Sample	Texamyl (g/l)	Color	Starch
1	0.5	Yellow + Green	No starch
2	1	Yellow + Green	No starch
3	2	Yellow + Green	No starch
4	3	Yellow + Little green	No starch
5	5	Yellow + Little green	No starch
6	8	Yellow	No starch

3.1.5 Determination of wet from air humidity

This is to determine how much water is present inside the fibers before desizing and after desizing as to know how much starch is left.

1. Cut 2 samples from 100% cotton and weigh them,
2. Dry the sample @ 105^0C at different times until the weight is constant

W_1 = 1.311g weight of the pot was 22.994g (WP)
W_2 = 1.284g weight of the pot was 23.631g

TABLE 16: DRYING OF SAMPLES TO CONSTANT WEIGHT @ 1050C

W_1 + Wp (g)	24.235	24.235	24.235	24.232	24.232
W_1 (g)	1.241	1.241	1.241	1.238	1.238
Time(mins)	15	30	45	60	75

Calculations
1.238g constant weight

1.311g…….100%
1.238g……x%
X =5.568%

Therefore wet = **5.568%**

3.1.6 Determination the amount of starch

Desizing – drying – weight the desized sample and absolutely dried sample

% starch = 1.311g-1.284g (1+5.568%/100%) *100
1.311g
=**-3.3%** (with air humidity)
Or

% starch = 1.311g (1-5.568%/100%) -1.238g *100
4(1-5.568%/100%)
= **-0.000093%** (without air humidity)

3.2 Testing No 2

3.2.1 Aim - To desize woven cotton (100%) in 5 minutes

(i) To compare the samples that have been desized with Texamyl (1-5) and the samples that has been desized by blank solution (6-10) as the recipe shown below:

Recipe

Desizing for 5 minutes	**"Scouring" for 5 min. in blank solution** (Without Texamyl)
(1. – 5. Sample)	(6. – 10. Sample)
Texamyl NU 5 g/l	~~Texamyl NU 5 g/l~~
Slovafol 909 1 g/l	Slovafol 909 1 g/l
T = 85° C	T = 85° C
pH 6, 5	pH 6, 5
NaCl 1 g/l	NaCl 1 g/l

(ii) Drying to constant weight – weight [g], T=95° C

(iii) Method:

Prepare the samples to be desized
Prepare dyebath using the above chemicals

Perform desizing @ 85^{0}C for 5 minutes
Check pH
Drying of samples
Check starch using a drop of iodine in each sample

(IV) Drying desized and boiled (in blank solution) samples to constant weight – weight [g]

(v)Calculation of weight-shortage

3.2.2 Results

TABLE 17: DRYING TO CONSTANT WEIGHT BEFORE DESIZING – WEIGHT [G], T=95° C

Time [min] / Sample	0	15	30	45	60	75	90	105	120	150	180	mark
1.	1,29845	1,24180	1,24005	1,23685	1,23445	1,23556	1,23304	1,23565	1,23455	1,23524	1,23455	W_1
2.	1,29625	1,24070	1,23955	1,23555	1,23605	1,23425	1,23477	1,23505	1,23438	1,23543	1,23428	W_2
3.	1,29509	1,23785	1,23465	1,23465	1,23345	1,23466	1,23255	1,23455	1,23382	1,23248	1,23248	W_3
4.	1,28299	1,22985	1,22865	1,22505	1,22445	1,22356	1,22336	1,22366	1,22405	1,22392	1,22258	W_4
5.	1,29295	1,23945	1,23805	1,23245	1,23188	1,23306	1,23155	1,23222	1,23382	1,23207	1,23208	W_5
6.	1,27825	1,22205	1,22155	1,21765	1,21945	1,21975	1,21735	1,21845	1,21687	1,21735	1,21658	W_6
7.	1,29515	1,24075	1,23855	1,23785	1,23632	1,23586	1,23678	1,23655	1,23602	1,23515	1,23488	W_7
8.	1,28065	1,22755	1,22745	1,22545	1,22258	1,22334	1,22298	1,22285	1,22324	1,22279	1,22285	W_8
9.	1,29669	1,24175	1,24005	1,23965	1,23689	1,23867	1,23815	1,23805	1,23854	1,23690	1,23748	W_9
10.	1,30255	1,24824	1,24355	1,24355	1,24325	1,24125	1,24295	1,24356	1,24218	1,24056	1,24158	W_{10}

TABLE 18: DRYING DESIZED AND BOILED (IN BLANK SOLUTION) SAMPLES TO CONSTANT WEIGHT – WEIGHT [G]

Time [min] / Sample	0	15	30	45	75	105	135	165	180	mark
1.	1,16812	1,14724	1,14125	1,14168	1,14289	1,14252	1,14192	1,14285	1,14068	W_{1ds}
2.	1,16435	1,14382	1,14256	1,14245	1,14358	1,14132	1,14126	1,14158	1,14098	W_{2ds}
3.	1,16275	1,14157	1,13925	1,13445	1,13919	1,13508	1,13625	1,13558	1,13588	W_{3ds}
4.	1,16008	1,13768	1,13226	1,13556	1,13275	1,13392	1,13192	1,13265	1,13285	W_{4ds}
5.	1,17052	1,14533	1,14217	1,14138	1,14059	1,14172	1,14268	1,14098	1,14082	W_{5ds}
6.	1,17206	1,14326	1,14172	1,14296	1,14255	1,14225	1,14322	1,14158	1,14152	W_{6b}
7.	1,18686	1,15556	1,15523	1,15631	1,15495	1,15465	1,15328	1,15385	1,15368	W_{7b}
8.	1,17924	1,14667	1,14572	1,14563	1,14638	1,14492	1,14635	1,14385	1,14450	W_{8b}
9.	1,19630	1,16097	1,16072	1,15914	1,15892	1,15842	1,15725	1,15685	1,15659	W_{9b}
10.	1,19476	1,16268	1,16132	1,16037	1,15894	1,15868	1,15935	1,16025	1,15970	W_{10b}

3.2.3 Calculation of weight-shortage

1. – 5. sample

$$\frac{w - w_{ds}}{w} * 100 = x\ \%$$

6. – 10. sample

$$\frac{w - w_b}{w} * 100 = y\ \%$$

TABLE 19: CALCULATION OF WEIGHT-SHORTAGE

	[g]		[g]		[%]		[g]		[g]		[%]
W_1	1,23455	W_{1ds}	1,14068	X_1	7,603580	W_6	1,21658	W_{6b}	1,14152	Y_1	6,169755
W_2	1,23428	W_{2ds}	1,14098	X_2	7,559063	W_7	1,23488	W_{7b}	1,15368	Y_2	6,575538
W_3	1,23248	W_{3ds}	1,13588	X_3	7,837855	W_8	1,22285	W_{8b}	1,14450	Y_3	6,407164
W_4	1,22258	W_{4ds}	1,13285	X_4	7,339397	W_9	1,23748	W_{9b}	1,15659	Y_4	6,536671
W_5	1,23208	W_{5ds}	1,14082	X_5	7,406987	W_{10}	1,24158	W_{10b}	1,15970	Y_5	6,594823
				$\bar{X}$	7,549376					$\bar{Y}$	6,456790

X % …weight-shortage starch

(= starch, residual fibers, impurities, oils)
some waxes and oils)

Y % …weight-shortage without starch

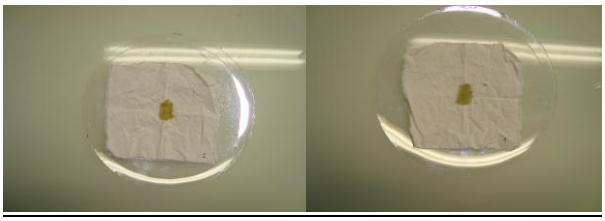
(= residual fibers, impurities, some waxes and

X % - Y % = Z %

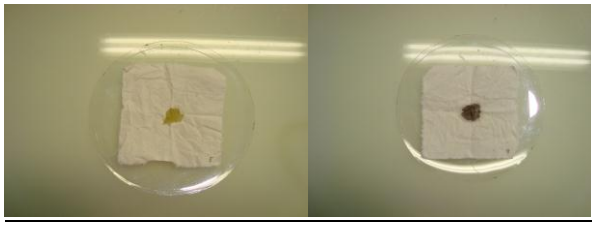
7,549376 - 6,456790 = **1,092586 %**

Z %... % pure starch was removed (% from absolutely dry sample)

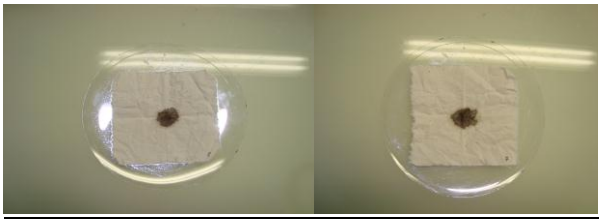
1 2



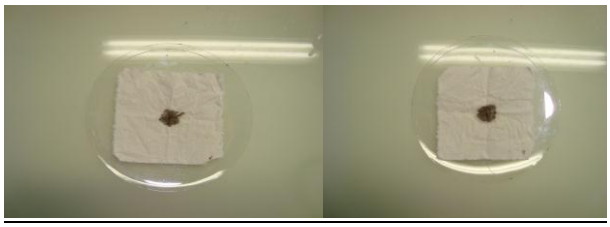
3 4



5 6



7 8



9 10

Fig 19: Samples 1-5 treated with Texamyl and samples 6-10 treated with blank solution for 5 minutes

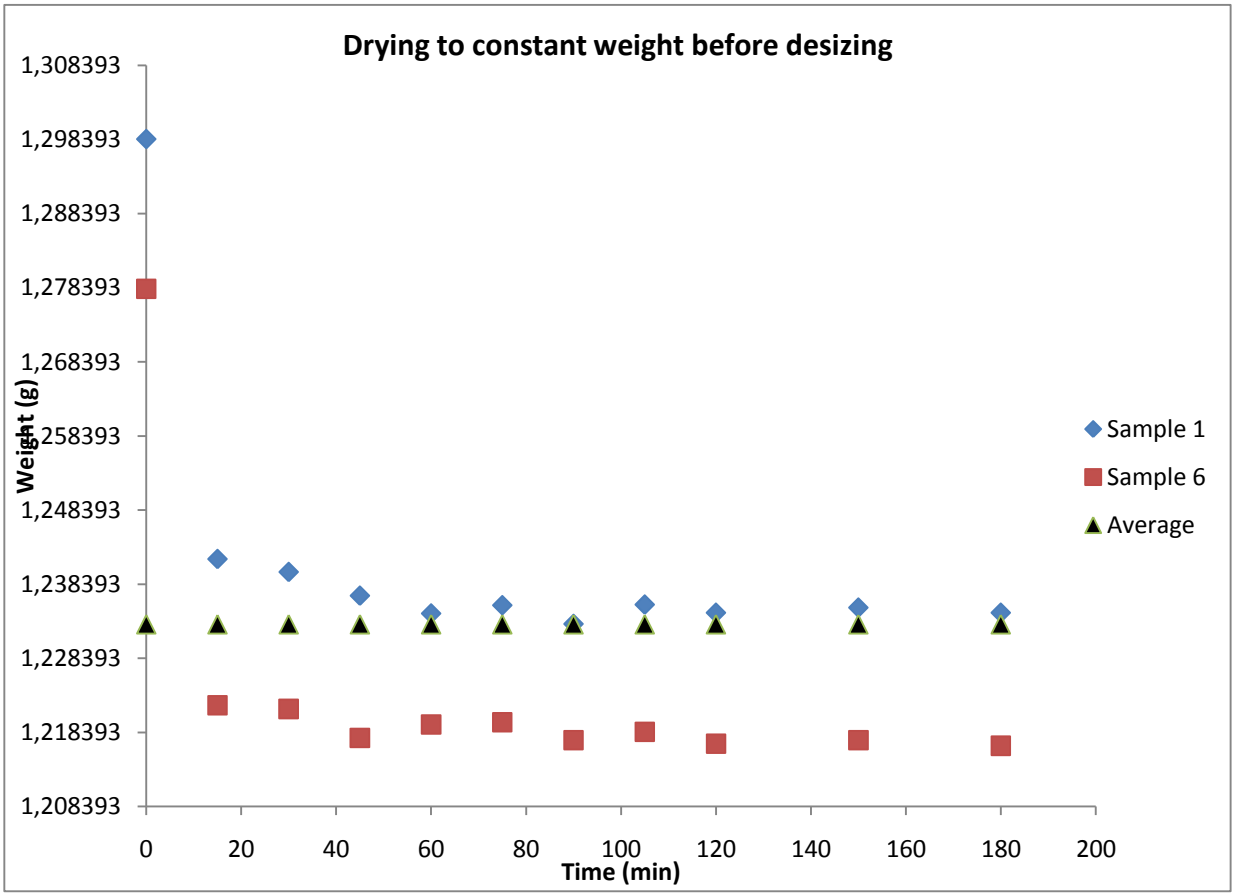


Fig 20: Drying of samples to constant weight @ 95^{0}C for 3hrs before desizing

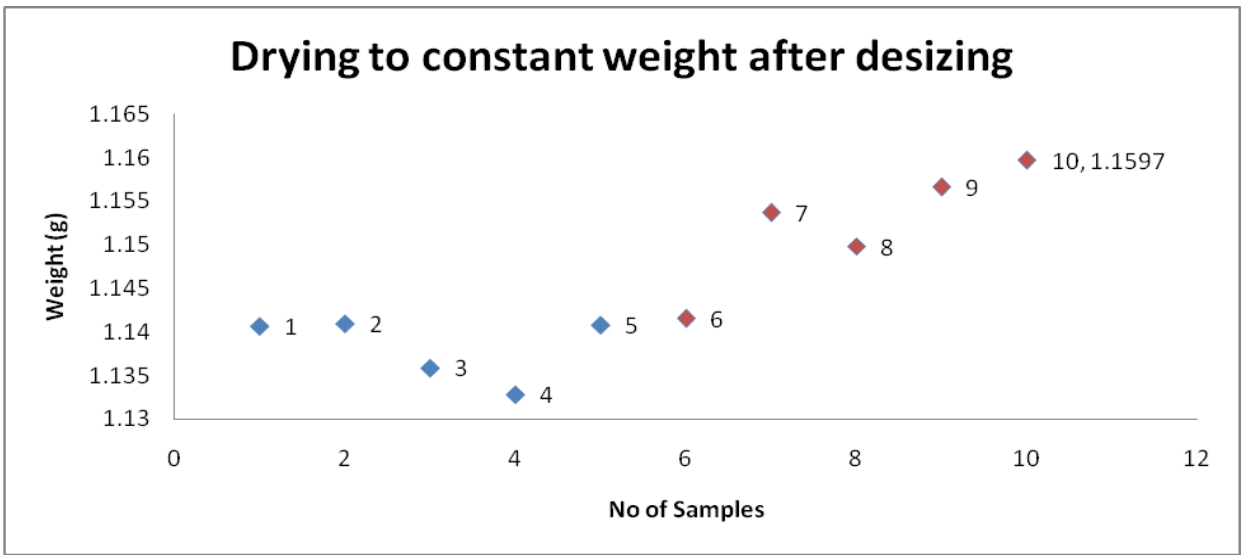


Fig 21: Drying of samples (1-5 & 6-10) to constant weight @ 95^{0}C after desizing for 5 minutes

3.3 Testing No 3

3.3.1 Aim – Desize of 100% woven cotton fabric for 20 minutes

The procedure was the same as Test No2; the only difference was the time. The time for experiment here was for **20 minutes**

3.3.2 Results

TABLE 20: DRYING TO CONSTANT WEIGHT – WEIGHT [G], T=95° C

Time [min] / Sample	0	30	60	90	120	150	180	mark
1.	1,30698	1,24485	1,24258	1,24312	1,24462	1,24387	1,24406	W_1
2.	1,29625	1,23586	1,23588	1,23482	1,23458	1,23527	1,23498	W_2
3.	1,30011	1,24077	1,24014	1,23985	1,24026	1,23931	1,23898	W_3
4.	1,29662	1,23816	1,23915	1,23702	1,23764	1,23791	1,23688	W_4
5.	1,29729	1,23688	1,23852	1,23795	1,23748	1,23692	1,23702	W_5
6.	1,28735	1,22988	1,22962	1,22854	1,22932	1,22792	1,22827	W_6
7.	1,30462	1,24756	1,24762	1,24650	1,24658	1,24682	1,24475	W_7
8.	1,28265	1,22595	1,22607	1,22315	1,22423	1,22398	1,22225	W_8
9.	1,29705	1,23945	1,24077	1,23838	1,23860	1,23764	1,23616	W_9
10.	1,29215	1,23475	1,23509	1,23158	1,23316	1,23143	1,23256	W_{10}

Desizing for 20 minutes

Texamyl NU	5 g/l
Slovafol 909	1 g/l
NaCl	1 g/l
pH	6,5
T =	85° C

1. – 5. Sample

"Scouring" for 20 min. in blank solution
(without Texamyl)

~~Texamyl NU~~	~~5 g/l~~
Slovafol 909	1 g/l
NaCl	1 g/l
pH	6,5
T =	85° C

6. – 10. Sample

TABLE 21: DRYING DESIZED AND BOILED (IN BLANK SOLUTION) SAMPLES TO CONSTANT WEIGHT – WEIGHT [G]

Time [min] / Sample	0	30	60	90	120	150	180	mark
1.	1,17368	1,15475	1,15317	1,15321	1,15298	1,15220	1,15262	W_{1ds}
2.	1,17352	1,14705	1,14557	1,14628	1,14597	1,14588	1,14548	W_{2ds}
3.	1,17762	1,15178	1,15078	1,15034	1,14986	1,14925	1,14872	W_{3ds}
4.	1,17818	1,15042	1,14936	1,14834	1,14906	1,14862	1,14792	W_{4ds}
5.	1,18402	1,14927	1,14827	1,14858	1,14747	1,14819	1,14775	W_{5ds}
6.	1,18562	1,15376	1,15163	1,15095	1,15148	1,15186	1,15077	W_{6b}
7.	1,20358	1,16786	1,16832	1,16838	1,16818	1,16686	1,16657	W_{7b}
8.	1,18406	1,14627	1,14675	1,14512	1,14568	1,14488	1,14508	W_{8b}
9.	1,19915	1,16218	1,16068	1,15968	1,16055	1,15910	1,15958	W_{9b}
10.	1,19474	1,15558	1,15472	1,15422	1,15406	1,15267	1,15185	W_{10b}

3.3.3 Calculation of weight-shortage

1. – 5. sample

$$\frac{w-w_{ds}}{w} * 100 = x\ \%$$

6. – 10. sample

$$\frac{w-w_b}{w} * 100 = y\ \%$$

TABLE 22: CALCULATION OF WEIGHT-SHORTAGE

	[g]		[g]		[%]
W_1	1,24406	W_{1ds}	1,15262	X_1	7,350128
W_2	1,23498	W_{2ds}	1,14548	X_2	7,247081
W_3	1,23898	W_{3ds}	1,14872	X_3	7,285025
W_4	1,23688	W_{4ds}	1,14792	X_4	7,192290
W_5	1,23702	W_{5ds}	1,14775	X_5	7,216537
				$\bar{X}$	7,258212

	[g]		[g]		[%]
W_6	1,22827	W_{6b}	1,15077	Y_1	6,309688
W_7	1,24475	W_{7b}	1,16657	Y_2	6,280779
W_8	1,22225	W_{8b}	1,14508	Y_3	6,313766
W_9	1,23616	W_{9b}	1,15958	Y_4	6,194991
W_{10}	1,23256	W_{10b}	1,15185	Y_5	6,548160
				$\bar{Y}$	6,329477

Calculation of weight-shortage

X % …weight-shortage

(= starch, residual fibers, impurities, waxes some waxes and oils)

Y % …weight-shortage without starch

(= residual fibers, impurities, some and oils)

X % - Y % = Z %

7,258212 – 6,329477 = **0,928735 %**

Z %... % pure starch was removed (% from absolutely dry sample)

Fig 22: Samples 1-5 treated with Texamyl (enzyme)

Fig 23: Samples 6-10 treated with blank solution

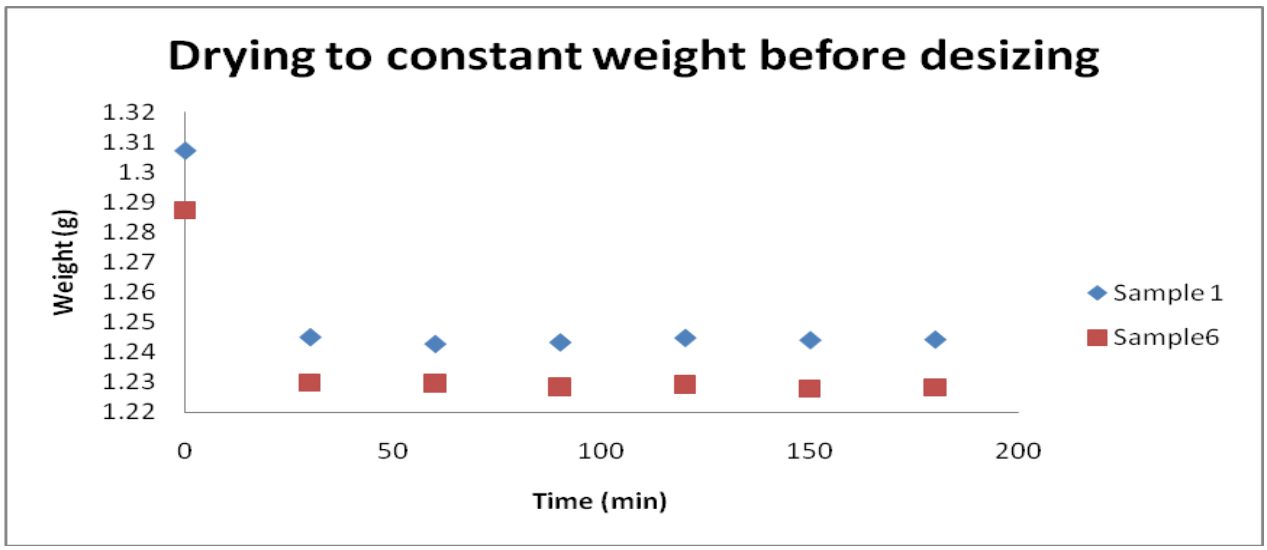


Fig 24 (a): Drying of samples to constant weight @ 95^{0}C for 3hrs before desizing

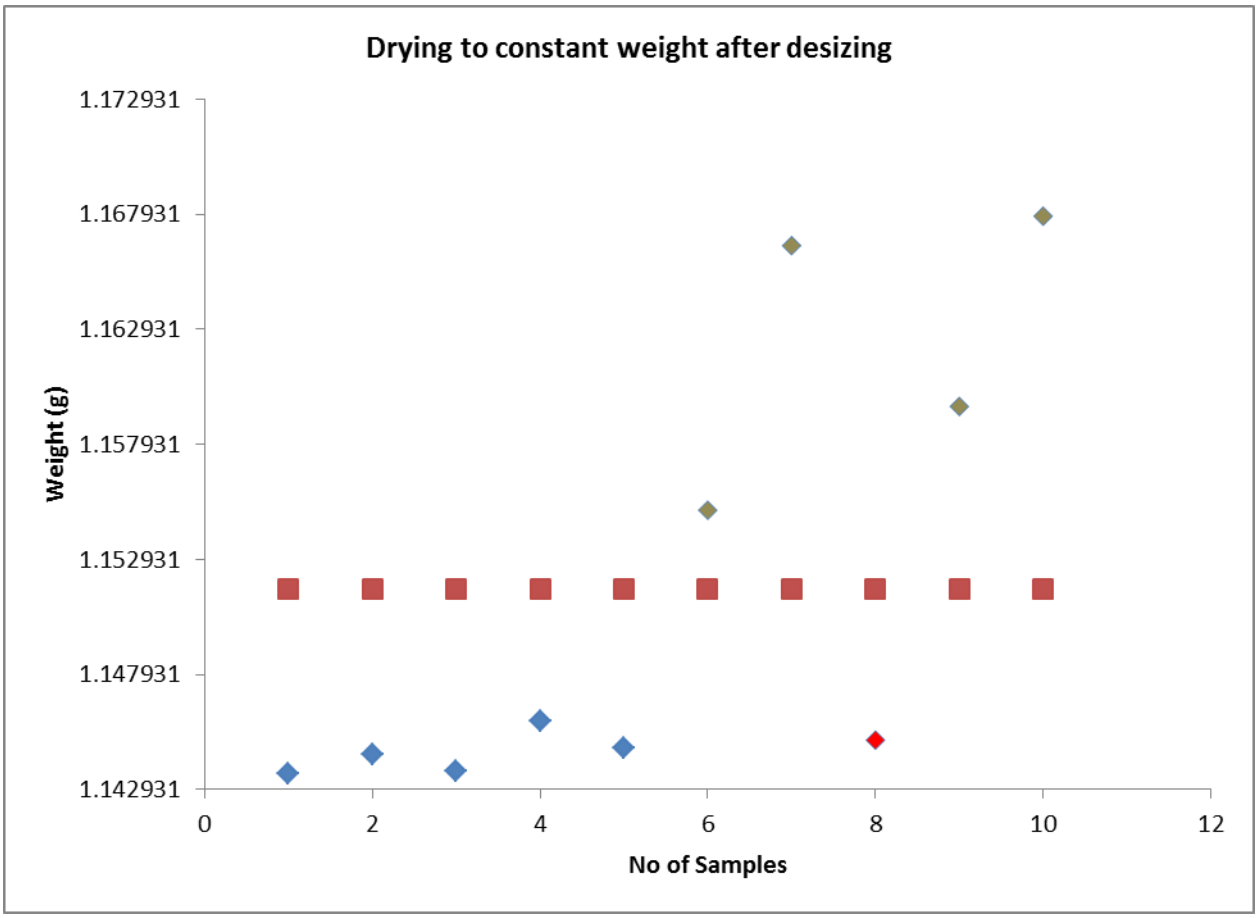


Fig 24 (b): Drying of samples (1-5 enzyme & 6-10 blank solution) to constant weight @ 95^{0}C after desizing for 20 min.

3.4 Testing No 4

3.4.1 Aim – To desize 100% woven cotton fabric for 60 minutes

The procedure was the same as Test No3; the only difference was the time. The time for experiment here was for **60 minutes**

3.4.2 Results

TABLE 23: DRYING TO CONSTANT WEIGHT – WEIGHT [G], T=95° C

Time [min] / Sample	0	30	60	90	120	150	180	mark
1.	1,27560	1,20592	1,20426	1,20465	1,20325	1,20423	1,20385	W_1
2.	1,26002	1,19285	1,19164	1,19200	1,19134	1,19055	1,19132	W_2
3.	1,26380	1,19608	1,19482	1,19586	1,19457	1,19468	1,19406	W_3
4.	1,25900	1,19285	1,19182	1,19208	1,19228	1,19168	1,19097	W_4
5.	1,27080	1,20468	1,20407	1,20445	1,20446	1,20384	1,20396	W_5
6.	1,28525	1,22093	1,22032	1,22057	1,21976	1,21975	1,21986	W_6
7.	1,30508	1,24026	1,23987	1,23947	1,23917	1,23944	1,23952	W_7
8.	1,27360	1,21186	1,21162	1,21096	1,21067	1,21124	1,20987	W_8
9.	1,28645	1,22376	1,22267	1,22197	1,22158	1,22257	1,22137	W_9
10.	1,27591	1,21348	1,21228	1,21238	1,21188	1,21305	1,21145	W_{10}

Desizing for 60 minutes solution

Texamyl NU	5 g/l
Slovafol 909	1 g/l
NaCl	1 g/l
pH	6,5
T =	85° C

1. – 5. Sample

"Scouring" for 60 min. in blank

(Without Texamyl)

~~Texamyl NU~~	~~5 g/l~~
Slovafol 909	1 g/l
NaCl	1 g/l
pH	6,5
T =	85° C

6. – 10. Sample

TABLE 24: DRYING DESIZED AND BOILED (IN BLANK SOLUTION) SAMPLES TO CONSTANT WEIGHT – WEIGHT [G]

Time [min] / Sample	0	30	60	90	120	150	180	mark
1.	1,17800	1,11788	1,11625	1,11736	1,11742	1,11616	1,11692	W_{1ds}
2.	1,16372	1,10508	1,10527	1,10577	1,10598	1,10512	1,10477	W_{2ds}
3.	1,16695	1,10957	1,10882	1,10926	1,10915	1,10887	1,10868	W_{3ds}
4.	1,16238	1,10502	1,10457	1,10502	1,10486	1,10482	1,10458	W_{4ds}
5.	1,17266	1,11727	1,11625	1,11654	1,11612	1,11610	1,11569	W_{5ds}
6.	1,19637	1,13938	1,13580	1,13926	1,13838	1,13807	1,13875	W_{6b}
7.	1,21220	1,15595	1,15527	1,15605	1,15554	1,15637	1,15534	W_{7b}
8.	1,18694	1,13062	1,13086	1,13112	1,13127	1,13116	1,13054	W_{8b}
9.	1,19892	1,14475	1,14249	1,14255	1,14238	1,14197	1,14208	W_{9b}
10.	1,18895	1,13285	1,13276	1,13289	1,13227	1,13318	1,13228	W_{10b}

3.4.3 Calculation of weight-shortage

1. – 5. sample

$$\frac{w-w_{ds}}{w} * 100 = x\ \%$$

6. – 10. sample

$$\frac{w-w_b}{w} * 100 = y\ \%$$

TABLE 25: CALCULATION OF WEIGHT-SHORTAGE

	[g]		[g]		[%]
W_1	1,20385	W_{1ds}	1,11692	X_1	7,220999
W_2	1,19132	W_{2ds}	1,10477	X_2	7,265051
W_3	1,19406	W_{3ds}	1,10868	X_3	7,150394
W_4	1,19097	W_{4ds}	1,10458	X_4	7,253751
W_5	1,20396	W_{5ds}	1,11569	X_5	7,331639
				$\bar{X}$	7,244367

	[g]		[g]		[%]
W_6	1,21986	W_{6b}	1,13875	Y_1	6,649124
W_7	1,23952	W_{7b}	1,15534	Y_2	6,791339
W_8	1,20987	W_{8b}	1,13054	Y_3	6,556903
W_9	1,22137	W_{9b}	1,14208	Y_4	6,491890
W_{10}	1,21145	W_{10b}	1,13228	Y_5	6,535144
				$\bar{Y}$	6,604880

Calculation of weight-shortage

X % …weight-shortage

(= starch, residual fibers, impurities, waxes some waxes and oils)

Y % …weight-shortage without starch

(= residual fibers, impurities, some and oils)

X % - Y % = Z %

7,244367 – 6,604880 **= 0,639487 %**

Z %... % pure starch was removed (% from absolutely dry sample)

Fig 25: Samples 1-5 treated with Texamyl only

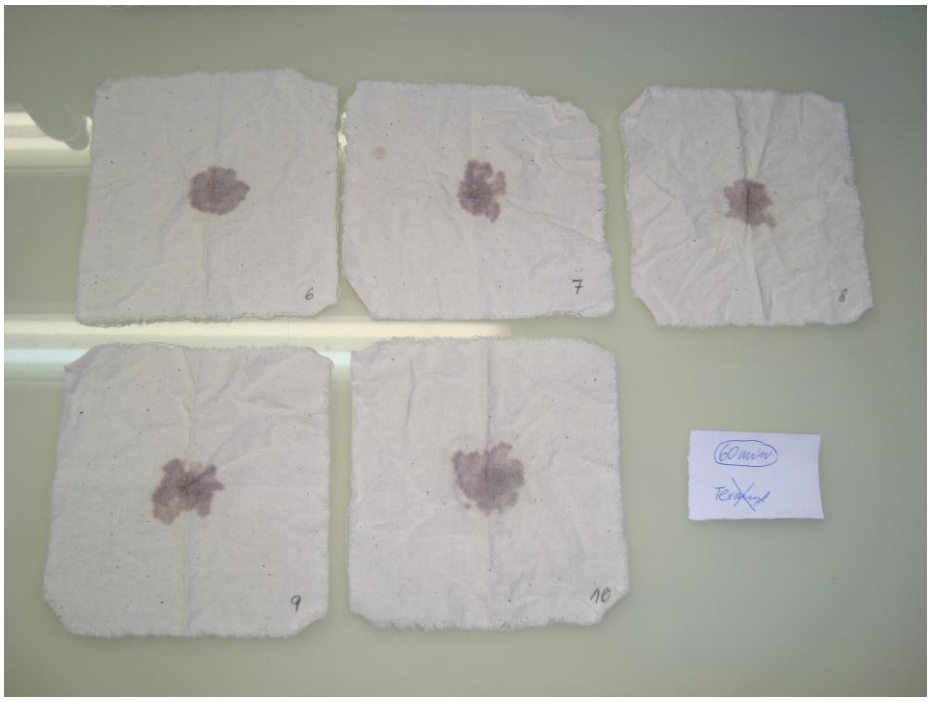


Fig 26: Samples 6-10 treated with blank solution

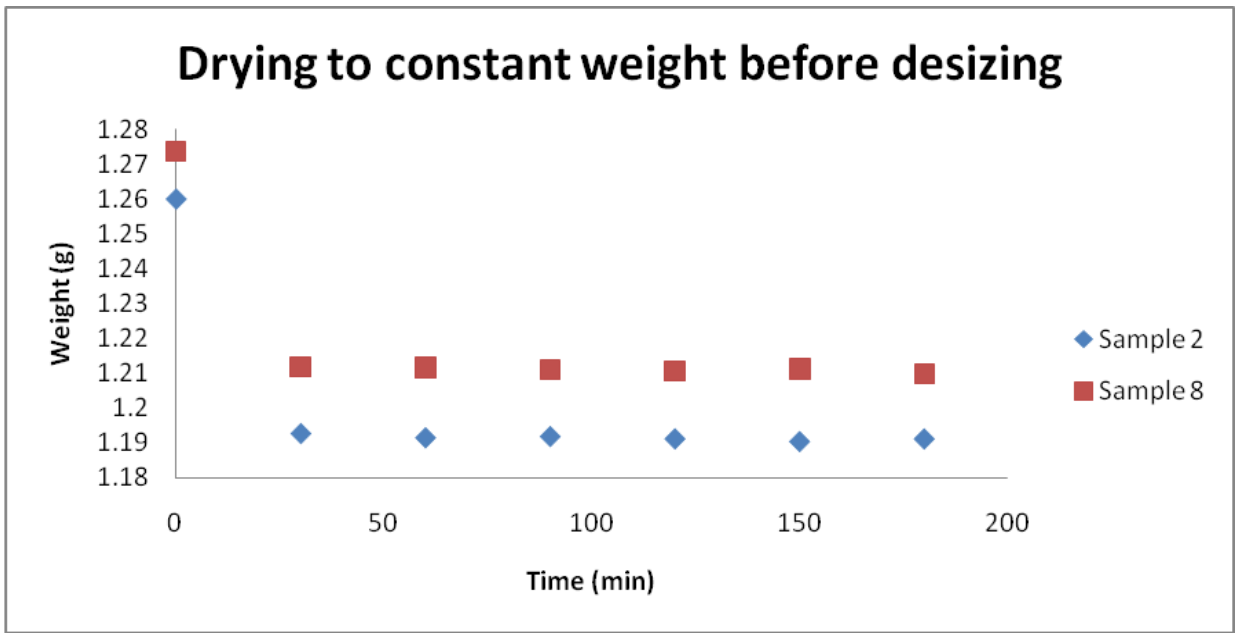


Fig 27 (a): Drying of samples to constant weight @ 95^{0}C for 3hrs before desizing

Fig 27 (b): Drying of samples (1-5 & 6-10) to constant weight @ 95^{0}C after desizing for 60 minutes

Time	X(mean)	Y(mean)	Z [%]
5	7.183839	5.818856	1.364983
20	7.258212	6.329477	0.928735
60	7.244367	6.604880	0.639487

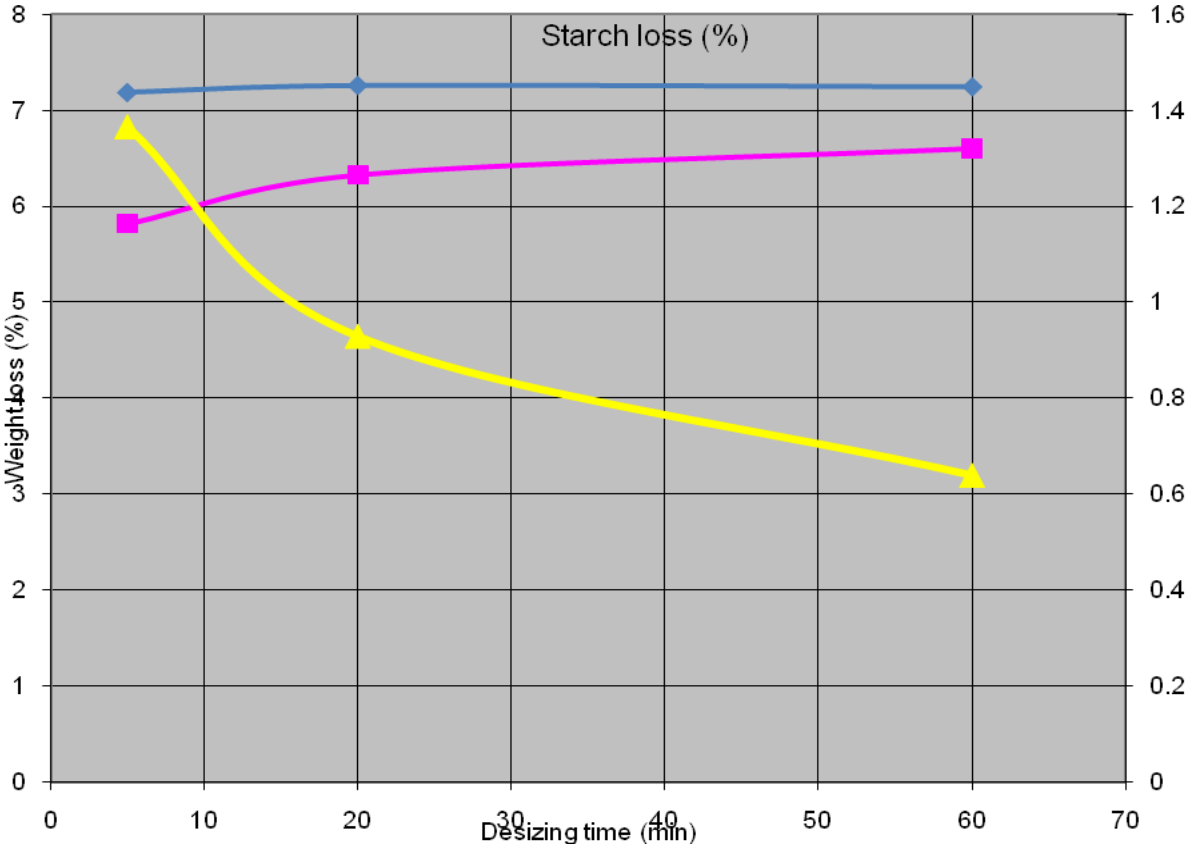


Fig 28: Relationship between X, Y, and Z for starch loss of 5 min, 20 min and 60 minutes

X (→) – Samples desized with Texamyl

Y (→) – Samples treated with blank solution

Z (→) – Total percentage of starch left in the sample from different times

3.5 Testing no 5 (100% Knitted Cotton)

3.5.1 Test for Starch for 5 minutes

(A) First Scouring the knitted fabric using the ff recipe:

Weight of fabric = 258.42g
Liquor ratio = 1:20
2--4 g/l Na_2CO_3
0.5--1 g/l Alfonal K (Wetting Agent)
1--2 g/l Syntapon (Detergent)
90 degrees Celsius for 30 minutes
Total Bath = 5 liters

(B) Dry the sample to constant weight

original weight = 246.22

weight(g)	236.34	233.08	232.22	231.58	231.57
time(min)	30	60	90	120	150

C) Sizing

Certain weight of the chemical mixture (starch) were added to anagitated cold water bath and stirring for 20 min. To wet the granules, then the temperature was raised to 80 degrees and cooked for 30 min. After cooking, the starch was fed to a padder containing the size solution (5%) @ 80^0C, run through the pad rolls to squeeze out the excess.
Size to wet pick-up of 100% and followed by drying @ ambient temperature for 24 hrs before testing.
Size to wet pick-up of 100% and followed by drying @ ambient temperature for 24 hrs before testing.

(D) Recipe for sizing and parameters

5% solid o.w.f	sample = 246.22g	starch = 12.311g
temp = 80	time = 30 min	pad = 71.6%
140g…….2l	250g……..100%	7kg……100l
12.5g…….xl	178.5g…….x%	xkg……2l
x = 178.5ml = 178.5g	**x = 17.428% (pick-up)**	**x = 0.14kg = 140g**

(D) Procedure (padder)

Weigh starch and water
Stir for 20 min @ 860 rpm (3,6Nm)
Boil @ 65-80degrees Celsius for 30 mins
Speed = 2-3m/min
Air pressure = 2bar
Pick-up = 70%

Pad and dry for 24 hrs

(E) Weigh the sample to constant weight

weight(g)	254.48	244.46	241.52	240.65	240.32	40.29	240.24
time(min)	0	30	60	90	120	150	180

3.5.2 Results

To calculate the amount of starch

231.57…….100%

240.24……X%

X = 103.74% STARCH = 3.74%

Cut 10 samples from the original sample 10*10cm and weigh them to constant weight

Time	0	30	60	90	120
Sample					
1	3.79369	3.59599	3.59444	3.59494	3.59486
2	3.82259	3.62366	3.66276	3.62486	3.62169
3	3.92515	3.71859	3.71469	3.71715	3.71720
4	3.60408	3.41356	3.41426	3.41276	3.41182
5	3.78227	3.58455	3.58405	3.58268	3.58266
6	3.79284	3.59147	3.59257	3.59165	3.59250
7	3.83942	3.63582	3.63704	3.63691	3.63686
8	3.73633	3.53610	3.53621	3.53527	3.53540
9	3.92351	3.71169	3.71223	3.71093	3.70484
10	3.82131	3.61504	3.61176	3.61284	3.60940

(F) Desizing using a new Chemical for 30mins @ 98degreees, pH 6-7 for 5 samples

Recipe :

Rucolase CML 200 0.5-1g/l

weight of sample = 3.6g

LR = 1:50

Total Bath = 180ml

Scouring with only water the other 5 samples (Blank solution)

Dry them in the oven and weigh to constant weight to calculate the amount of starch

Time	0	30	60	90	120
Sample					
1	3.65534	3.45765	3.45659	3.45456	3.45461
2	3.68328	3.48736	3.48605	3.48637	3.48638
3	3.74831	3.54702	3.54778	3.54679	3.54685
4	3.43912	3.25376	3.25406	3.25562	3.25560
5	3.62707	3.43495	3.43475	3.43472	3.43471
6	3.71126	3.50761	3.50838	3.50891	3.50938
7	3.83424	3.62591	3.62380	3.62353	3.62355
8	3.77700	3.56836	3.56833	3.56930	3.56952
9	3.65353	3.45048	3.45188	3.45169	3.45170
10	3.72101	3.52042	3.51918	3.51865	3.51870

3.5.3 Calculation of weight shortage using the following formula:

$$\frac{W - W_{ds}}{W} * 100 = x\ \%$$

W1 =before desized

W2 = after desized

		%
1	3.90%	x
2	3.70%	x
3	4.50%	x
4	4.57%	x
5	4.10%	x
6	2.30%	y
7	0.37%	y
8	-0.96%	y
9	6.80%	y
10	2.50%	y

Calculation for pure starch (pure starch z %)

X% - Y% = Z%

X% = 4.15%

Y% = 2.20%

Z% = 1.95%

3.6 Testing No 6

3.6.1 Suction Capacity Determination

Aim

To check the suction capacity of material after desizing in different times: 0; 5; 20 & 60 minutes.

Materials

Cut 4 samples, size 190*10mm

Sized fabric – the original sample	sample 1
Desized sample for 5 minutes	sample 2
Desized sample for 20 minutes	sample 3
Desized sample for 60 minutes	sample 4

Method

1. Cut the samples to equal length
2. Prepare a solution of dye or chromium salt
3. Insert the samples in a solution as follows:

Fig 29: Samples dipped in a dye

4. Wait for 30 minutes and dry samples thoroughly
5. Observe and check the results

Results

TABLE 26: SHOW RESULTS AFTER 3 SUCTION CAPACITY DETERMINATION TESTS

Time	0	5	20	60
Height (cm) / 30 minutes	10	52	75	93
	9	64	70	72
	10	65	90	86
AVG	9.667	60.333	78.333	83.667
STDEV	0.577	7.234	10.408	10.693
CV (%)	5.973	11.990	13.287	12.780

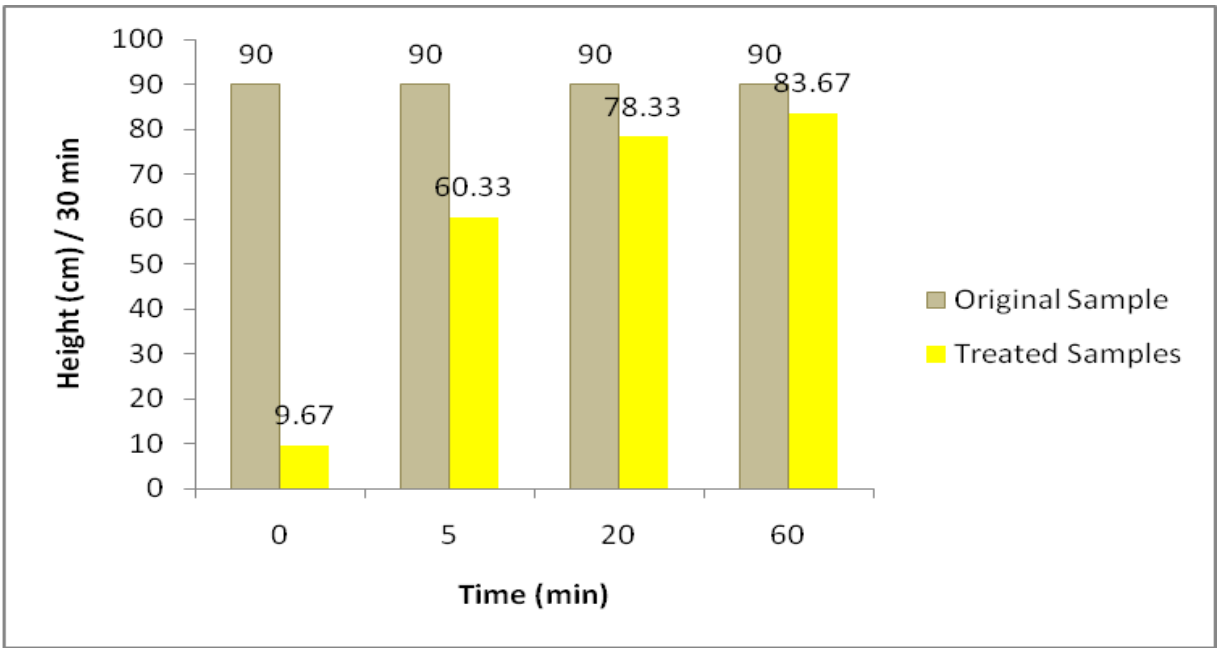


Fig 30: Results for 3 suction capacity determination tests

The figure above shows that the more time for desizing, the higher the suction capacity is determined. It also support the theory which mentioned that, energy and costs are reduced compared to oxidative desizing.

3.7 Testing No7

3.7.1 CIE Whiteness

Aim

To compare the untreated cotton (with starch and impurities) with treated cotton (enzyme & blank solution). Figure 31, shows the results using the ***D65 /10^0*** machines.

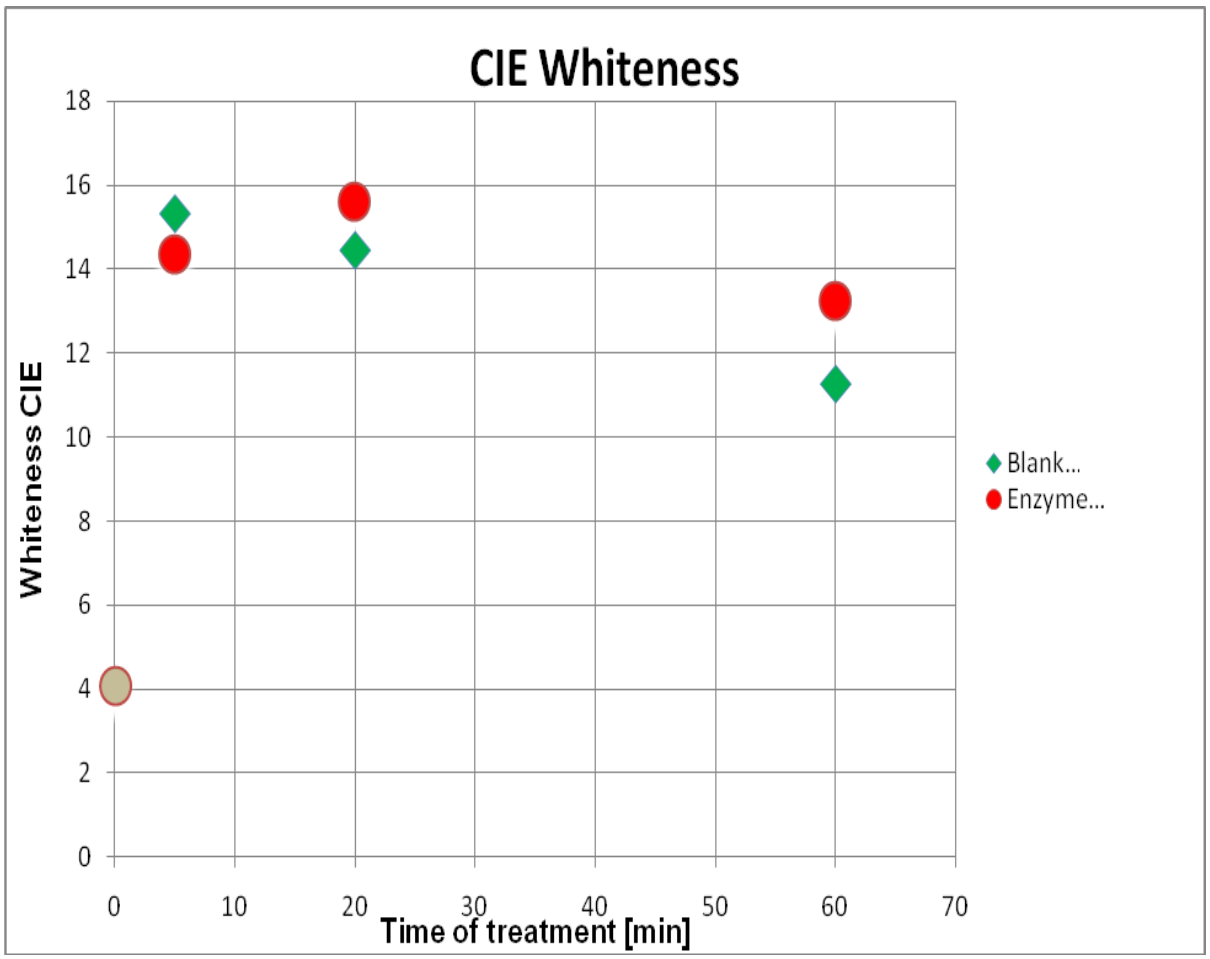


Fig. 31: Results of CIE whiteness in treated (enzyme & blank solution) and untreated samples

3.7.2 CIE Lab co-ordinates

AIM

It is designed to approximate human vision. It aspires to perceptual uniformity and its L component closely matches human perception of lightness. The 3 co-ordinates of CIELAB represents the lightness of the colour (L=0 yields black and L = 100 indicate diffuse white) [27].

A* - its position between rd/magenta and green, (-) values represent green and (+) values represent magenta.
B* - (-) values indicate blue and positive values indicate yellow
Figure 32, shows the results of the samples in terms of CIELAB co-ordinates

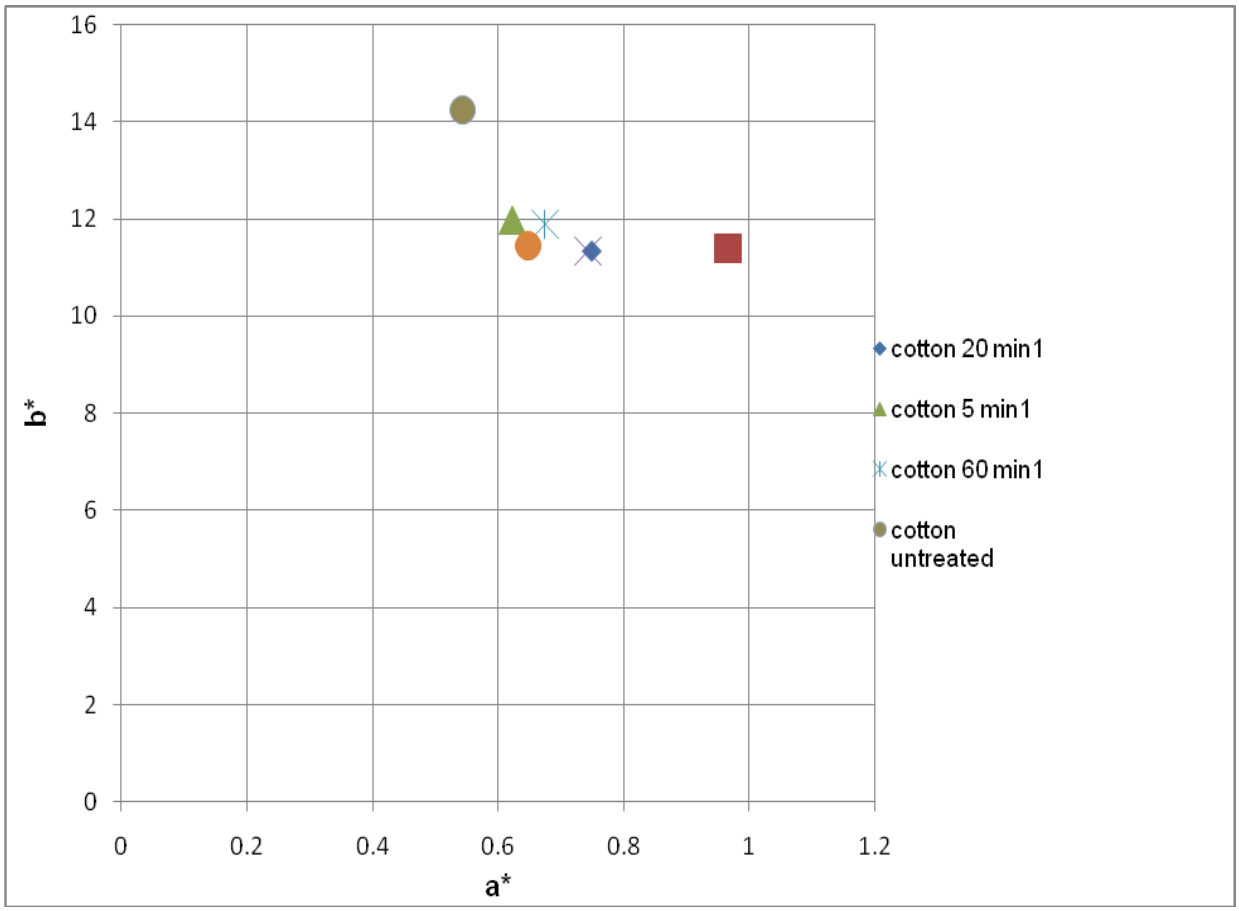


Fig 32: CIEL*a*b* co-ordinates

3.7.3 Lightness and Chroma

AIM

To determine the relationship between lightness and chroma from treated (enzyme & blank solution) and untreated sample. Figure 33 shows the results as follows:

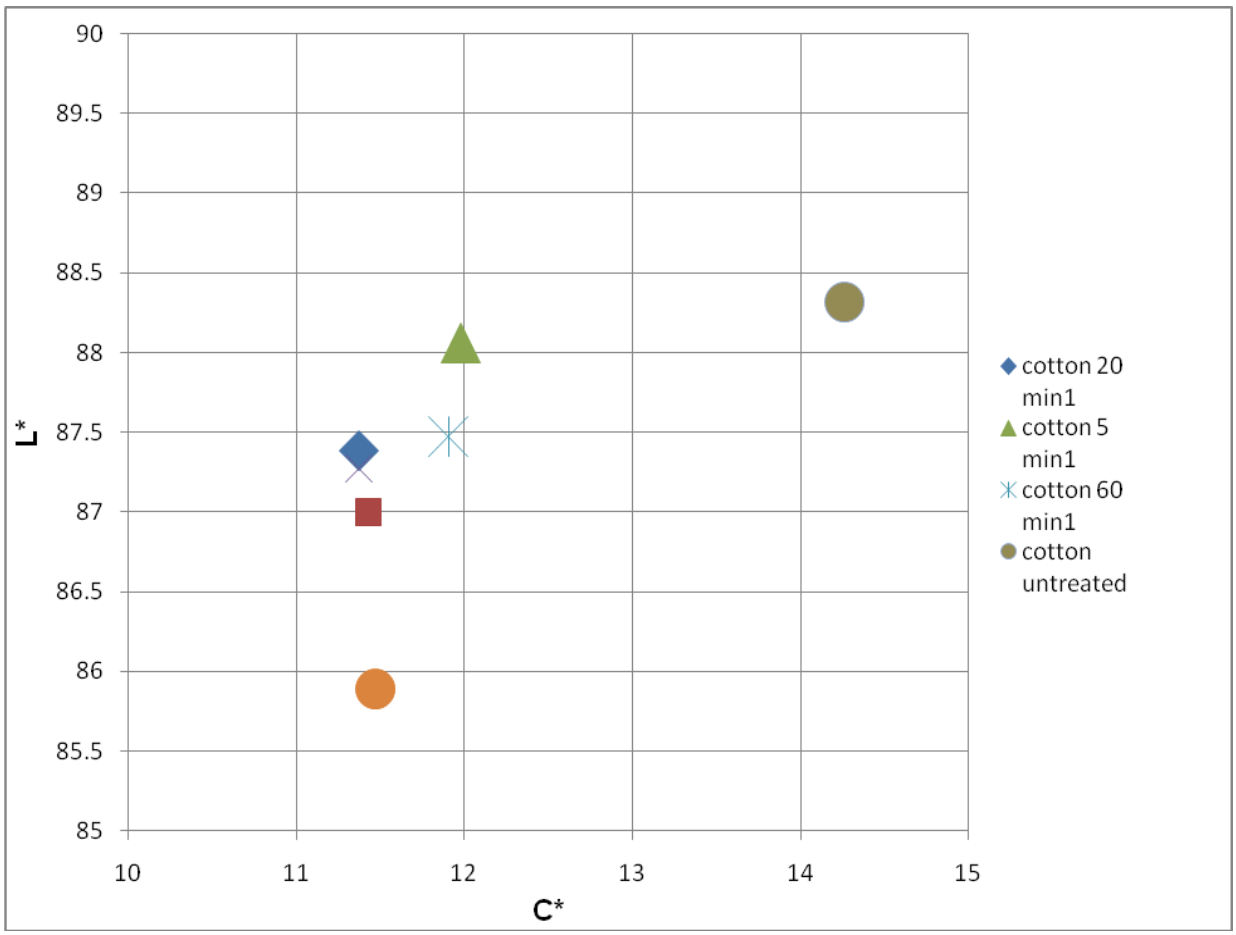


Fig 33: Relationship between lightness (L) and Chroma (C)

3.7.4 CIE XYZ

AIM

The chromaticity diagram represents pure colours on the periphery, i.e. the mono-chromatic radiation corresponding to the colours of the spectrum, indicated by the wave length. The line connecting the ends of the diagram is known as the line of purples, because it corresponds to the colour purple. The achromatic colours (white, greys, and blacks) are shown at the centre of the colour space. Figure 34 and figure 35 shows the original chromaticity graph and graph with results respectively:

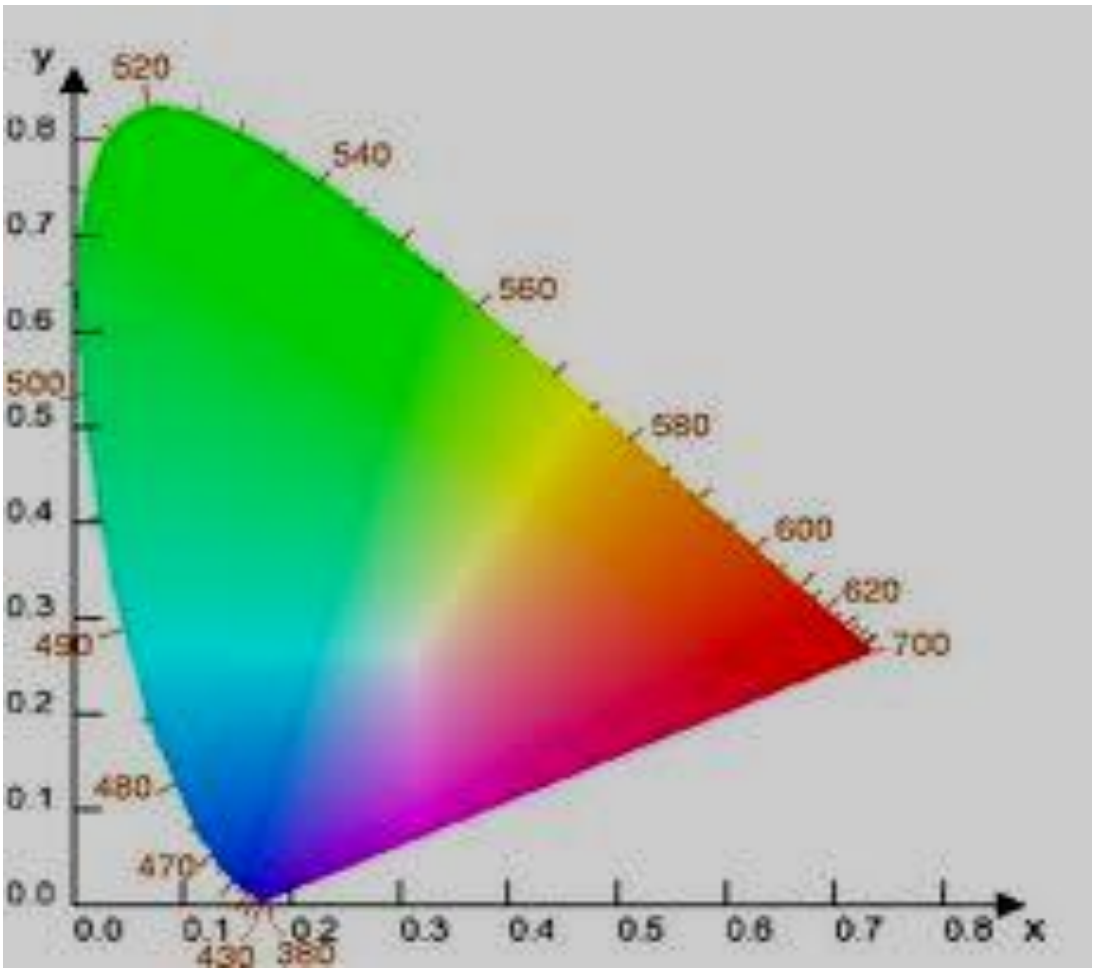


Fig 34: CIE 1931 Chromaticity diagram

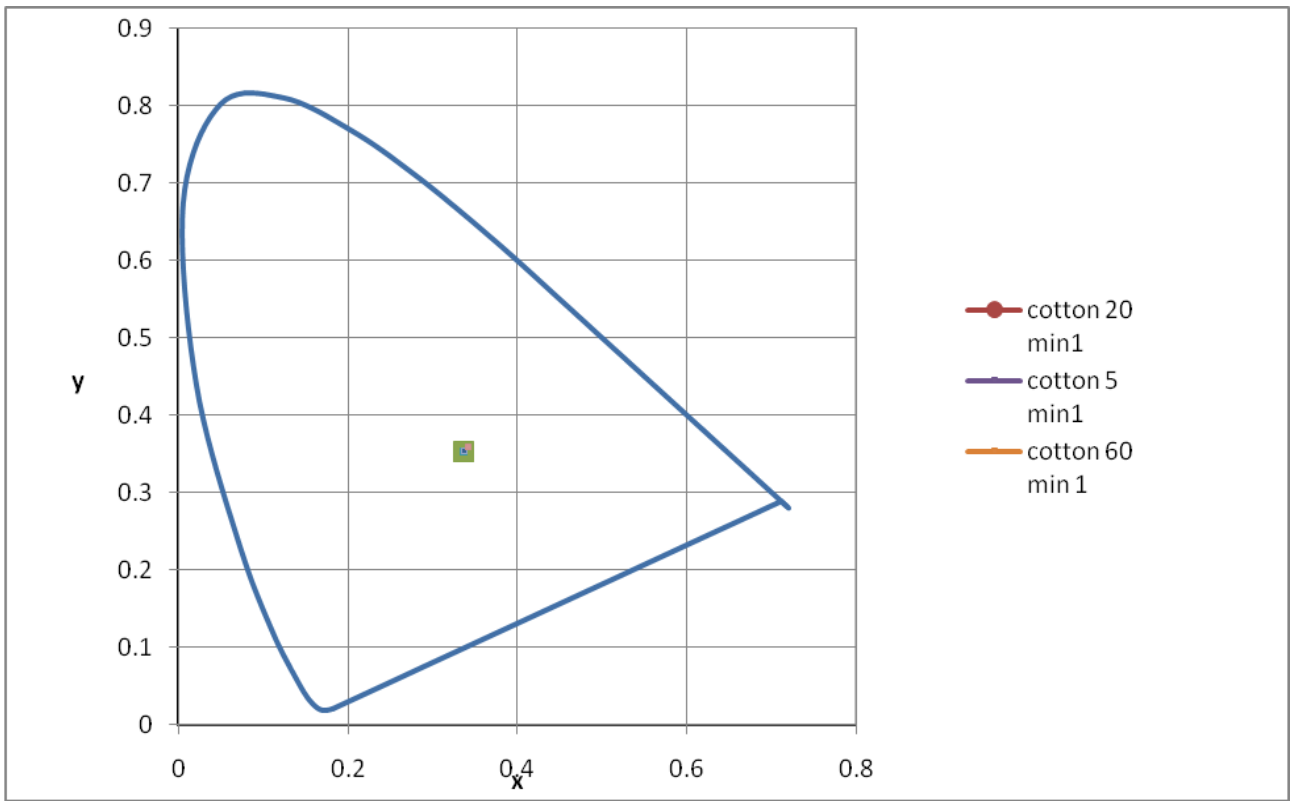


Fig 35: Results of samples as per CIE: The standard color space, as determined by the ''standard observer'' model, colour gamut

3.7.5 Auto-fluorescence of starch

AIM

Simple method of analysis the amount of starch left on the fabric after desizing beside calculations, Iodine, suction capacity determination etc. Fig 36, shows the starch left on the fabric ''visually'', using a UV light.

MATERIALS

Samples and UV torch (light) only.

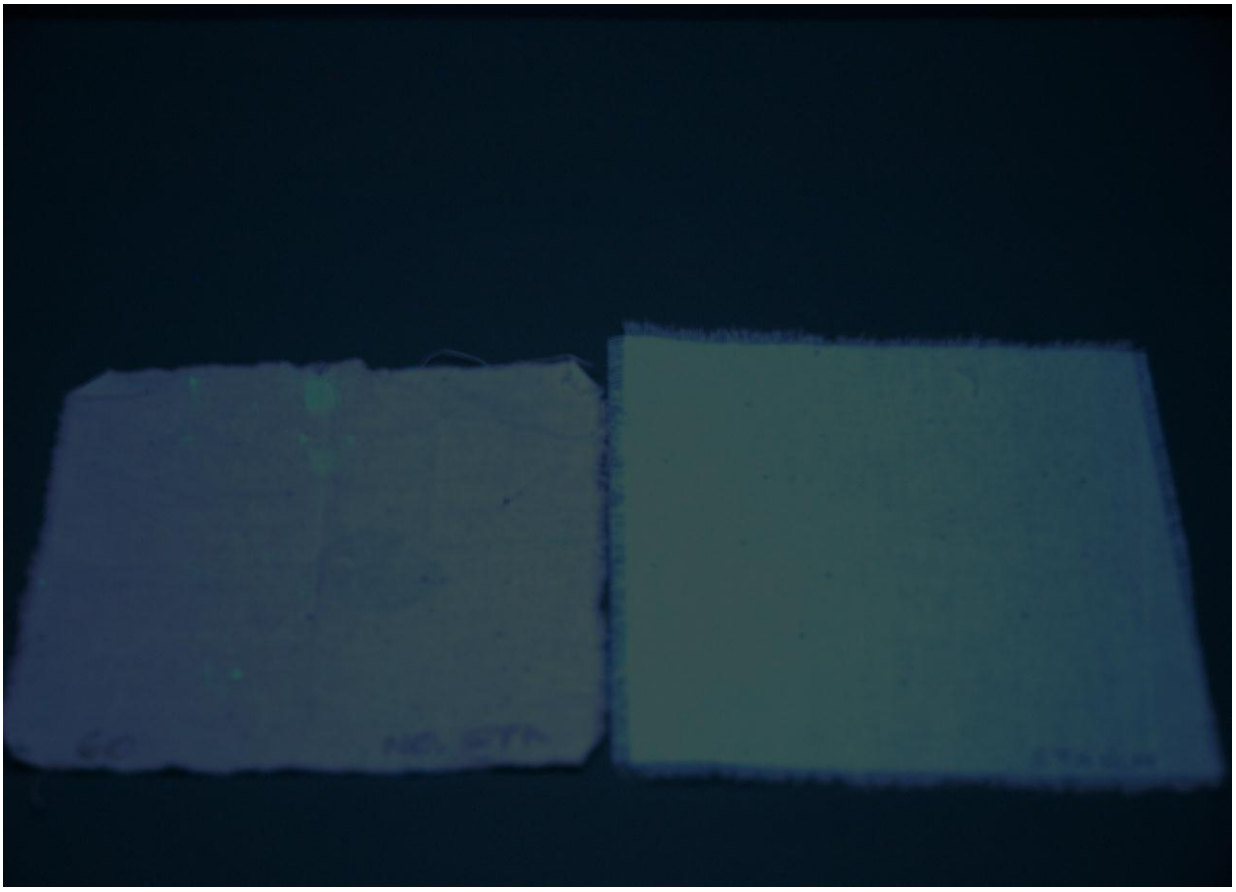


Fig 36: Treated sample (white spots) and untreated sample (brighter) under UV light

4 Discussion

4.1 Desizing:

There are basically three types of desizing methods that are being adopted according to the fabric construction, end-use purpose and the economical requirements:

1) Acid desizing
2) Basic desizing and
3) Enzyme desizing

The last two treatments are still being followed and the first one has been slowly going out of usage due to fabric strength loss as we know that the cotton is sensitive to acids. The purpose of desizing is to remove the sizing agents such as starches, gums, PVA etc. The gums and PVA can be removed by simple hot wash (for synthetic fabrics). But starch has to be degraded into smaller water soluble molecules by the process of hydrolysis. The hydrolysis of starch using enzymes under particular concentration, temperature and duration is called **desizing.**

4.2 For different amount of enzymes and different pH

- From experiment No1, the aim was to desize the fabric at different pH's of 3; 5; 8 & 10 @ 85^0C for 30 minutes and the results shows that from pH 5 to pH 10 the starch is degradable. After that a neutral pH (pH 7) was used of which it also degrades starch and it has been recommended.
- For desizing using different amount of enzymes (Texamyl) to desize the fabric: 0.5, 1, 2, 3, 5 & 8 g/l, for all these concentrations the results were positive (starch was degraded). Using concentration of 1-5g/l is also recommended for 100% woven and knitted cotton.

Let us look at the comparisons between conventional desizing and enzyme based desizing recipes:

4.2.1 Hydrogen Peroxide desizing (oxidative method/Basic method):

Caustic soda 100%	= 40 g/l	Temperature of bath	= room temperature
Sandozol HS	= 0.3 g/l	pH of bath	= 10 to 10.5
Hydrogen Peroxide 50%	= 3.3 g/l	pH of the fabric	= 8.5
Stabilizer AWNI	= 0.3 g/l	Nip Pressure	= 2.2.kg/sq.cm
Pick up	= 70%	Speed	= 100 meters/minute

4.2.2 Enzyme Based desizing:

Texamyl (enzyme)	= 1-5 g/l
Bath temperature	= 80°C

Common salt (NaCl) = 1 g/l
pH of bath = 6.5 (different pH)
Slovafol (nonionic wetting agent) = 1g/l
Liquor ratio = 1:100

4.3 Desizing of 100% woven cotton for 5, 20 and 60 minutes

Methods of desizing were followed using different times, 5 minutes, 20 minutes and 60 minutes. The results were conducted by the use of Iodine solution to check the presence of starch for different times and all were positive (starch was degraded) and the following figure shows the summary of the results:

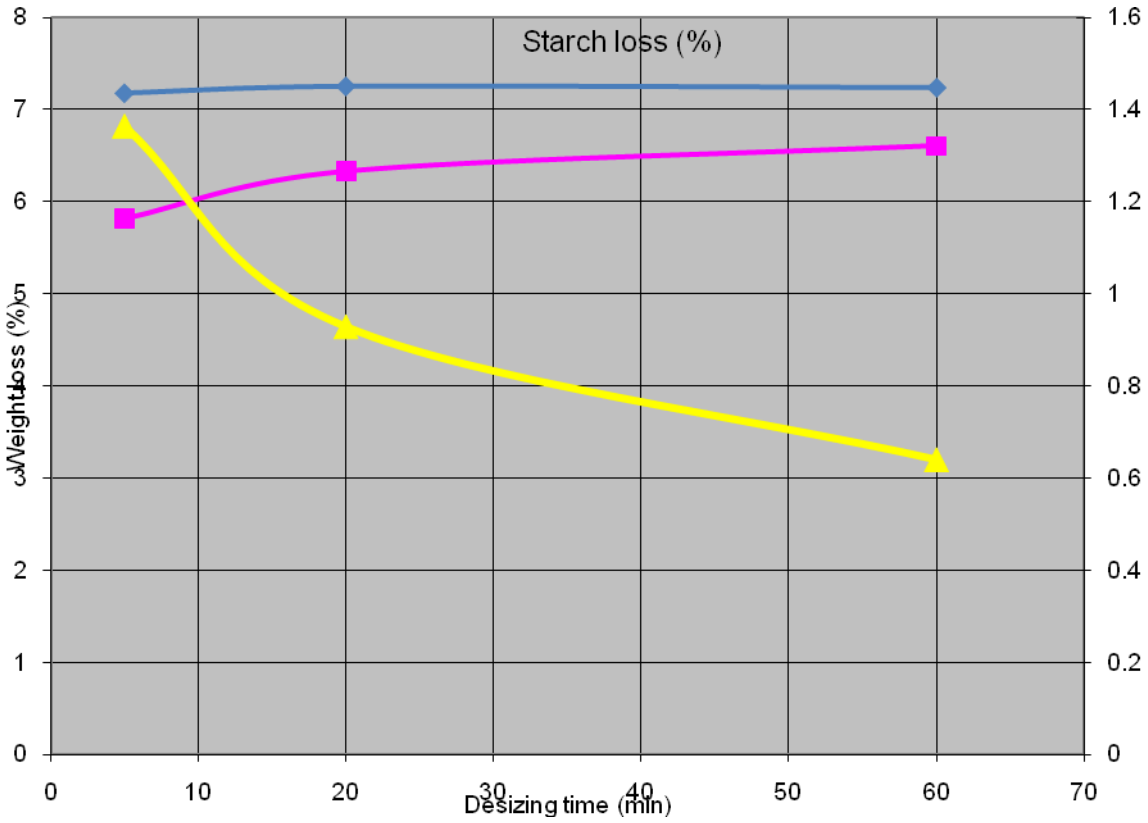


Fig 37: Relationship between X, Y, and Z for starch loss of 5 min, 20 min and 60 minutes

X (→) – Samples desized with Texamyl

Y (→) – Samples treated with blank solution

Z (→) – Total percentage of starch left in the sample from different times

X - The results shows that, the more time we desize the more the starch loss % increases.
Y – The weight loss (%) also increases on samples that were boiled in blank solution but not as much as with X, there is still more starch that needs to be degraded.

Z – The total amount of starch that is left on the fabric as the time increase as follows:
For 5 minutes = 1.36%
For 20 minutes = 0.93%
For 60 minutes = 0.64%

NB: These results are calculated on the Experiments and Results Chapter.

4.4 Suction Capacity Determination

The aim was to check the suction capacity of material after desizing in different times: 0; 5; 20 & 60 minutes. The results showed us that the more time for desizing, the higher the suction capacity is determined. It also support the theory which mentioned that, energy and costs are reduced compared to oxidative desizing.

4.5 CIE Whiteness

The whiteness of the untreated and treated fabrics (for different times) was tested using $D65/10^0$ and results showed us that, the more time we desize the higher the value of whiteness.

4.6 CIELAB co-ordinates

''The co-ordinates of the Hunter 1948 L, a, b. However, *Lab* is now more often used as an informal abbreviation for the CIE 1976(L*, a*, b*) color space''. The L* value was 88 so the sample was lighter (L=100, white). The co-ordinates a* and b* had positive values and that shows us that the sample from a* values was magenta and for b* values was yellow and that gives us an overall results of red sample.

4.7 Lightness and Chroma

The relationship between chroma and lightness from the results shows that, the untreated sample had too much chroma and the treated samples had less chroma and lighter than untreated sample. This is due to the facts that, the untreated sample have impurities and too much starch so the chroma is higher.

4.8 Auto-florescence of Starch

This is the simple method of analyzing the amount of starch left on the fabric with the use of UV lamp. The results also showed us that there are some small particles that are left on the fabric due to inadequate desizing conditions. The untreated sample appears to be brighter than treated sample because there is a lot of starch which absorbs UV light and the treated sample appear to be dull.

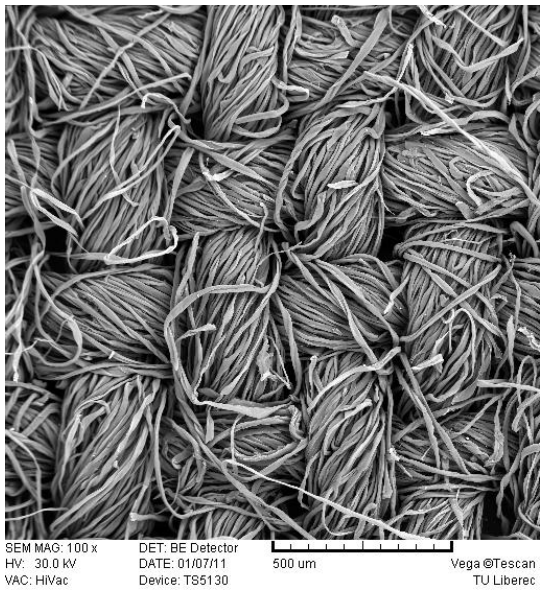
5 Conclusion and Future Trends

There is a huge potential to reduce energy costs in the pre-treatment processes in a textile mill. For example, the process of bleaching needs to be addressed in future in order to reduce energy costs and enhance the quality of bleached fabric. Enzymes could provide the answer. It should be mentioned that the vast majority of enzyme applications today are used for the
treatment of cotton. The textile industry is also looking for new ways to deal with current problems on fibres other than cotton. This includes finding ways to improve the quality of the so-called bast fibres (hemp, linen, etc.), wool and even synthetic fibres. The search is on for industrial enzymes that are commercially viable for use on these substrates.
There is also a need to find alternative ways of dealing with an array of different sizing agents other than starch. Enzymes could even be used in future to break down dyestuffs in the effluents from dye houses and denim finishing laundries. Dyestuffs remain one of the most difficult substances to remove at an effluent treatment plant. With all these opportunities and the need to move towards sustainable development, industrial enzymes could provide some of the solutions the textile industry is looking for in the future.

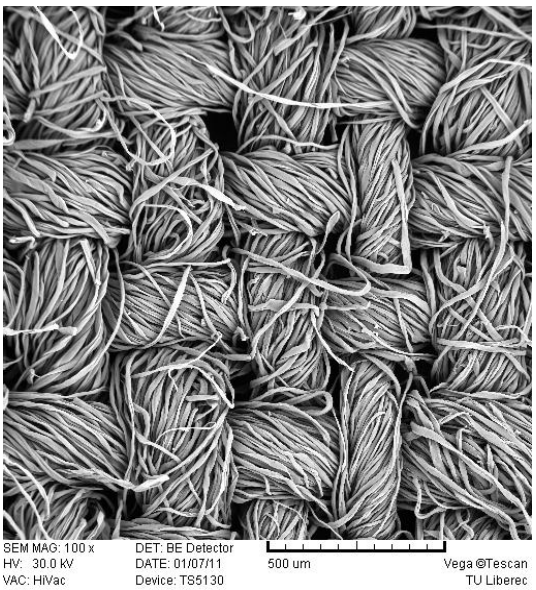
For enzymatic desizing, compared to conventional desizing, there is a huge difference in terms of energy used, the chemicals used, damage of fabric etc. According to the research and the results, enzymatic treatment does not damage fabric, does not reduce fabric strength, reduces energy or power consumption and is environmental friendly of which it is the best thing for human health.

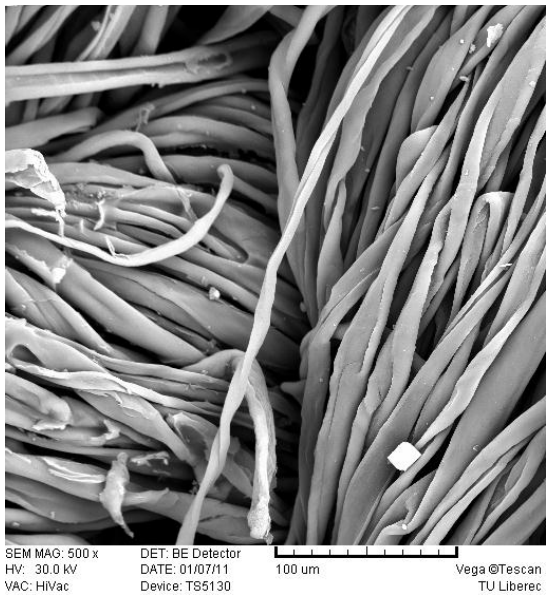
6 Analysis of the fabric surface before and after desizing

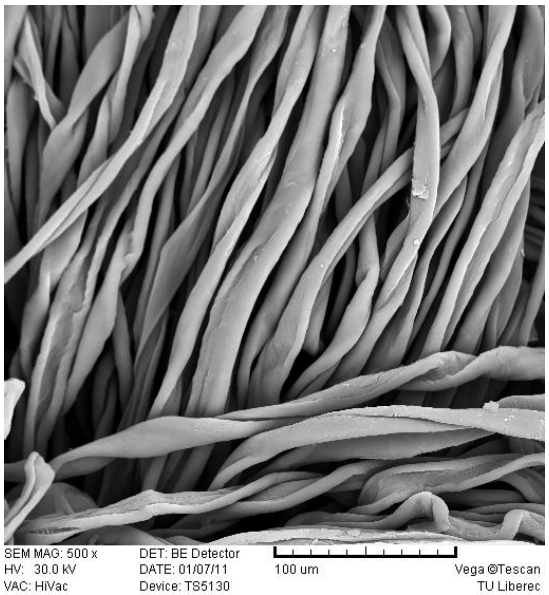
Before treatment

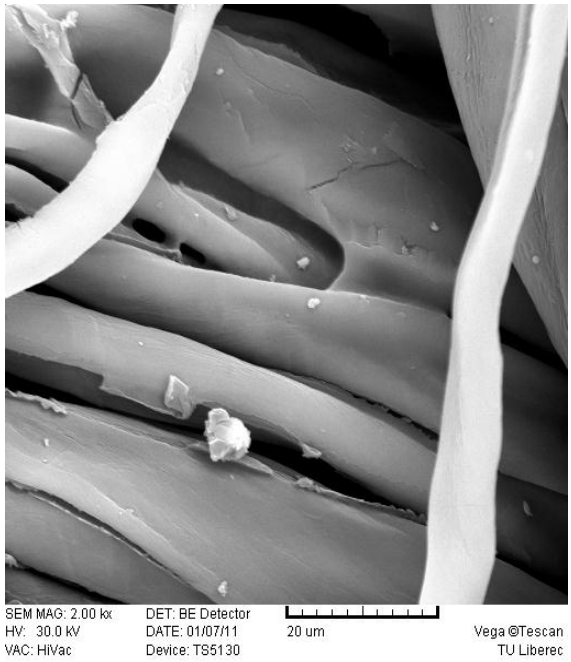


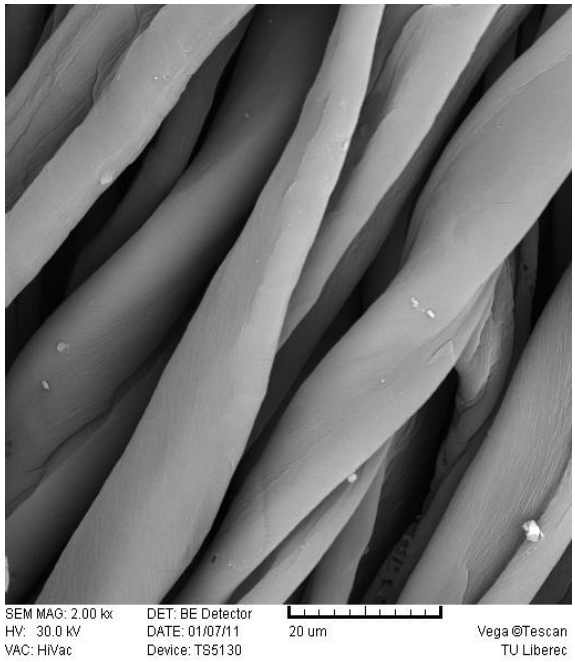
After treatment

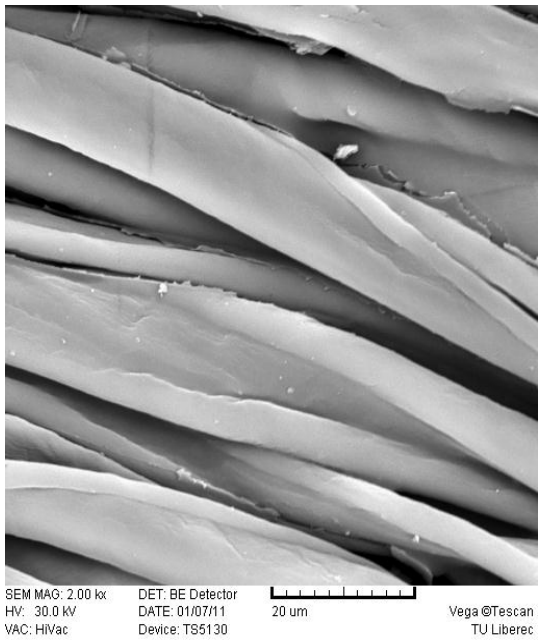


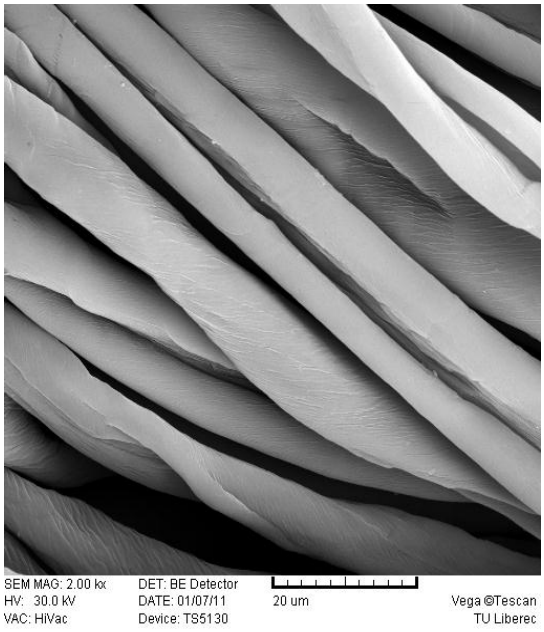












The above samples were treated for 20 minutes by enzymatic desizing and the results after electro-microscopy shows that there is no or very few white spots that shows the presence of starch. The white spots on the ''before treatment'' samples shows the starch. There is also no fabric damage or strength loss of fabric compared to oxidative desizing. Only smooth surface of which is advantageous to fabric handle and for further processes.

7 Abbreviations:

ABTS	2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) – chemical compound used to observe the reaction kinetics of specific enzyme.
ATP	Adenosine-5'-triphosphate (is a multifunctional nucleotide used in cells as a coenzyme)
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
Cu	Copper
Ea	Activation Energy
EC	Enzyme Commission
EIS	Enzyme Inhibitor Substrate (complex)
EPR	Electron Paramagnetic Resonance
ES	Enzyme Substrate
HOBT	1- Hydroxybenzotriazole (common synthetic mediators)
kDA	kilodalton-is a unit used for indicating mass on an atomic or molecular scale
MSDS	Material Safety Data Sheet
NAD	nicotinamide–adenine dinucleotide
NADH	nicotinamide–adenine dinucleotide, reduced
NADP	nicotinamide–adenine dinucleotide phosphate
NHPI	N-hydroxyphthalimide (common synthetic mediators)
P	Products
RNA	ribonucleic-acid
RP	Redox Potential- is a measure of the tendency of a chemical species to acquire electrons and thereby be reduced
GOx	Glucose oxidase

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